INFLUENCE OF PHOSPHORUS LOADING ON BIOGEOCHEMICAL CYCLING OF NITROGEN IN NORTHERN EVERGLADES SOILS

By

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Major Department: Soil and Water Science

The northern Everglades ecosystem has been affected by phosphorus (P) loading from agricultural drainage waters resulting in a distinct eutrophic gradient with high total soil P in areas proximal to surface water inflow points. In addition, the vegetative community has shifted from the natural sawgrass (*Cladium, spp*)-open water system to dense, monotypic cattail (*Typha, spp.*) affecting ecosystem function. These effects were distinctly seen in the Water Conservation Area 2A (WCA-2A) of the Everglades.

I sought to investigate the influence of P on the biogeochemical cycling of nitrogen (N) along the eutrophic gradient. The availability and cycling of N in wetlands

can affect ecosystem productivity and water quality. Rates of organic N mineralization, nitrification, and denitrification were measured on soil collected from three depths at eight sites along eutrophic gradient. A series of laboratory and field studies were conducted to discern the influence of P on the biogeochemical cycling of N.

Organic N mineralization was significantly increased with P additions in both short term (days) and long term (months) studies. Aerobic mineralization rates were two times higher than rates measured under anaerobic conditions. The size/activity of the microbial pool was significantly increased by P loading, leading to an increase in inorganic N release. Initial nitrification rates appeared to be regulated by the availability of oxygen, while potential rates were influenced both by the microbial pool size and substrate concentration. The activity of denitrifying enzymes in the soil was found to be influenced by nitrate concentrations. However, denitrifying potential appeared to be higher in soils with high total P content.

The results of laboratory and field studies indicate that mineralization, or net release of inorganic N is affected by P availability to the microbial pool. Potential rates of denitrification also appeared to increase with increasing P concentration, however only after the NO_3^- limitation was exceeded. The increased concentrations of NH_4^+ may provide a stimulatory effect on the growth of macrophytes in the wetland. Greater macrophyte growth may then alter both ecosystem structure and function by continually cycling N back into organic forms (macrophytes) and re-releasing the inorganic N through decomposition.

CHAPTER 1
INTRODUCTION

Global Nitrogen Cycle

There are several large global pools of N, however turnover times can be quite long, limiting availability to organisms. Ninety eight percent of the earth's N is locked up in igneous rocks and is effectively out of contact with the biosphere. The atmosphere above the land is comprised of 78% N_2 and is an enormous reservoir of this necessary life sustaining element. Considering a hectare of soil and using 0.15 % N as an average for cultivated soils, there might exist about 7 Mg of N in the soil while the air above that hectare might contain ~300,000 Mg of N as N_2 (Brady and Weil, 1996).

Dinitrogen gas contains a triple bond, resulting in a very stable and inert gaseous state. The ability to break the triple bond, converting N_2 to a readily utilized form by the majority of plants is restricted to a relatively few genera of symbiotic and non-symbiotic microorganisms. Therefore these microorganisms provide an essential conduit, tapping the atmospheric reservoir of N, making it available to a host of other organisms.

Microorganisms are also responsible for converting organic N to inorganic forms, which are available to plants. Still other microorganisms are responsible for removing N from terrestrial and aquatic ecosystems and returning it back to the atmosphere as N_2 gas. Consequently, the biogeochemical cycling of N is important within a particular

ecosystem, metered by microbial communities, to help maintain productivity and sustainability over time.

Nitrogen exists in various oxidation states ranging from –3 to +5 and consequently can be found in one form or another, in every ecological niche. In soils, the majority (95-99%) of N exists in organic forms, which are largely unavailable to higher plants. Organic N in soils is comprised of proteins, amino acids, and as components of humic substances. Ammonification is the transformation of organic N to an inorganic form (NH_4^+) occurring under both aerobic and anaerobic conditions. Nitrification, an obligate aerobic process, is the autolithotrophic conversion of NH_4^+ to NO_3^-. Denitrification is the reduction of NO_3^- to N_2 gas under anaerobic conditions. The juxtaposition of both aerobic and anaerobic zones in soils makes it possible for all these processes to occur simultaneously and sequentially within a relatively restricted soil volume.

Over time, an ecosystem will evolve a specific biological composition including soils microorganisms, macrophytes and animal species dependent primarily upon resource allocation. The biogeochemical cycling of nutrients is generally controlled at the lowest trophic levels. Species of plants adapt to the nutrient status of the system or are replaced by physiologically superior competitors. Nutrient availability and subsequent transfer of nutrients up the food chain determines the carrying capacity of the ecosystem, which may remain stable over extended periods. Any significant perturbation, which throws off the established available ratio of nutrients or changes the level of resources available, can throw an ecosystem into imbalance.

Statement of the Problem

The Florida Everglades has been described by Davis (1943), as a vast, organic-rich peat wetland, which once encompassed 10,000 km^2 stretching from the fringes of Lake Okeechobee, south and west to the shoreline of Florida Bay. Excess water drained out of Lake Okeechobee over a shallow sill and moved by overland sheet flow through an extensive sawgrass marsh.

The first comprehensive study of the Everglades ecosystem entitled "The Natural Features of Southern Florida; Especially the Vegetation and the Everglades" was published by the Florida Geological Survey (Davis, 1943). Drainage maps included in the text depict the already completed Hillsborough, North New River, West Palm Beach and Miami Canals which comprise the major drainage system of the Everglades Agricultural Area (EAA).

The Water Conservation Areas (WCAs) were constructed in the early 1960's by the South Florida Water Management District (SFWMD) and consisted of surrounding large tracts of sawgrass marsh south of the EAA by earthen dikes and installing water control structures within the dikes. The WCAs were delineated by the aforementioned network of drainage canals leading from the EAA.

The Everglades was classified as a Class III water body by the State of Florida in 1994. This designation as such, requires that water quality is maintained to insure populations of native flora and fauna are not thrown into imbalance (McCormick and O'Dell, 1996). Under prompt of this statute, the SFWMD has conducted and funded several research projects to investigate and document both the natural and impacted Everglades system. Research has focused on investigating soil characteristics and

processes regulating water quality to better understand the influence of nutrient loading on ecosystem structure and function.

There exist three major ecosystem compartments, in contact with the water column, where research has focused on discerning the impact of P loading, and include macrophyte communities, periphyton communities and recently deposited peat soil. Sawgrass (*Cladium jamaicense*) was found to be specifically adapted for a low nutrient environment and, with little competition, existed in nearly monotypic stands covering the majority of the historical Everglades basin (Davis, 1991). The rate of cattail (*Typha sp.*) expansion in WCA-2A has increased from 1 % y^{-1} in 1971 to 4 % y^{-1} in 1987 (Wu et al., 1997). Sawgrass was found incapable of modifying the allocation of biomass in response to soil total P concentrations, however, cattail exhibited a change in biomass allocation dependent on soil total P (Miao and Sklar, 1998). There was a 60:40 biomass allocation (root:leaf) for cattail in low nutrient environments, which switched to 40:60 (root:leaf) allocation in high nutrient environments, perhaps leading to a competitive advantage for space and light, crowding out the natural sawgrass vegetation.

Periphyton biomass, fertilized with P, increased in field plots established within the WCAs, while no net increase in biomass was seen in those plots fertilized with only N (Flora et al., 1988; Vymazal et al., 1994). Additionally, periphyton contained elevated total P tissue concentrations in areas of elevated soil total P, while still dominated by sawgrass suggesting periphyton tissue concentration might be used an early indicator of change in ecosystem nutrient status (McCormick and O'Dell 1996).

The most extensive work to date conducted on the characterization of soil properties, focused on soil P forms (Koch and Reddy, 1992; Craft and Richardson, 1993;

Reddy et al., 1993; DeBusk et al., 1994; Craft and Richarson, 1997; and Reddy et al., 1998). These studies have documented elevated soil total P concentrations closest to surface water inflow points coincident with the spread and dominance of the invasive cattail. Davis (1991) warned that continual P loading could lead to increased P levels downstream as the assimilatory capacity of the cattail was exceeded. Comparisons of total P soil concentrations from 1990-91 with data collected in 1996-97 suggests the P enrichment "front" has moved several kilometers over 6 y, into areas still dominated by sawgrass today (Reddy et al., 1999).

The microbial biomass of surface soil and detritus can be the most reactive portion of the soil and therefore might respond quickly to changes in nutrient availability. The size of the microbial biomass pool in detritus was found to be highest at stations closest to the inflow (DeBusk and Reddy, 1998). This research suggests that soil characteristics of recently accreted soil/detritus can be diagnostically stronger and an earlier indicator of ecosystem impact or change than simply noting changes in macrophytes communities.

There has been limited research conducted on the influence of P loading on the organic C and N mineralization rates of soils. Early work on volcanic soils sought to examine the relationship between organic C and N mineralization in glucose and P amended soils (Munevar and Wollum, 1977). Significantly greater CO_2 evolution occurred when both P and glucose were added together than by glucose additions alone. Organic N mineralization was increased by inorganic N additions at the highest P addition level leading the authors to conclude that P was the major limiting nutrient to

microbial activity while N limited microbial activity only as P became non-limiting (Munevar and Wollum, 1977).

There has been very little research on the influence of P enrichment on other biogeochemical nutrient cycles in wetland soils. One recent investigation explored the effects of P enrichment on organic matter turnover in the Everglades and found potential organic C mineralization rates of soil were significantly correlated with substrate P concentrations in soil (DeBusk and Reddy, 1998). Organic C mineralization was strongly influenced by P content in standing dead and detrital material due to high C availability. However, P content had little influence over C mineralization at depth as C availability due to high lignin content, became limiting in buried peat. The authors also noted that microbial biomass C as a percent of total C decreased with increasing distance from the inflow and was significantly correlated with soil TP. Consequently, P enrichment can significantly increase the turnover rate of C, and data suggested the increased size of the microbial pool is associated with increased mineralization rates (DeBusk 1996).

Peat soils from the Everglades National Park were studied to determine the effects of C:P ratio on C mineralization (Amador and Jones, 1997). Over the range of C:P ratios ranging from 2000 – 200, CO_2 evolution increased for a given moisture content of the soil. A similar result was observed for CH_4 evolution. In addition, the authors noted water content exhibited some control on C mineralization, leading to the conclusion that moisture content might be a significant factor for C mineralization as P becomes less limiting (Amador and Jones, 1997).

Objectives and Scope of Research

The overall goal of this study was to determine the influence of P loading on the wetland biogeochemical cycling of N with possible links to ecosystem vegetation changes. The study area provided an excellent opportunity to look at long term (10's of years) effects of continual nutrient loading on soil processes regulating nutrient availability and water quality. Research was conducted along a 10 km P-enrichment gradient consisting of an impacted region with a high level of allochthonous nutrient inputs at the north end and a more natural system dominated by closed-cycling of nutrients and an oligotrophic ecosystem status at the south end.

In order to investigate whether P loading to an oligotrophic wetland (P-limited) has elevated microbial populations and catabolic activities, the following key questions needed to be addressed: (i) What is the influence of P loading on mineralization of organic N?; (ii) How do various inorganic electron acceptors influence organic N mineralization?; (iii) What is the influence of P loading on the coupled processes of nitrification-denitrification?; and (iv) What is the influence of P enrichment on turnover and overall N loss from the system?

The overall hypothesis of the study was: P loading to an oligotrophic wetland has accelerated N cycling processes in soil, resulting in increased availability of inorganic N and higher rates of N loss from the system. Specific hypotheses are:

1. Phosphorus loading increases microbial populations, decreases N:P ratios, and increases organic N turnover.
2. Increased rates of organic N mineralization provide a direct feedback mechanism by increasing the supply of inorganic N to microbes and vegetation.

3. Nitrate loss through denitrification is regulated by NO_3-N concentrations, rather than by dissolved organic C or P availability.
4. Phosphorus enrichment has increased N loss through coupled nitrification/denitrification reactions.

The specific objectives of the proposed study were to determine the influence of P loading on; (1) potential ammonification rates of soil and detritus under aerobic and anaerobic conditions, (2) potential nitrification rates of soil and detritus, (3) denitrifying potential and activity of the denitrifying enzyme of soil and detritus. The final objective was to construct a nitrogen budget for soils and identify the limiting process regulating overall N loss.

Dissertation Format

Chapter 1 provides an introduction for the overall dissertation, including a statement of the problem addressed, a list of specific research objectives, and a brief review of literature limited in scope to Everglades eutrophication. Chapters 2-6 are formatted as complete manuscript works intended for subsequent publication with each containing appropriate literature citations for the specified process(es) discussed. Chapter 2 presents research on the influence of P on the release of inorganic N correlated to the microbial biomass of wetland soil in field and laboratory experiments. Chapter 3 documents potential rates of net N mineralization under dominance of various inorganic electron acceptors. Chapter 4 investigates the initial and potential nitrification rates and coupled potential denitrification rates that mediate inorganic N removal from wetlands.

Chapter 5 explores the effect of P and N loading on the distribution of denitrifying enzymes in soil, documenting potential removal rates of N from the aquatic system. Chapter 6 links together the various N storages and transformation processes producing a simple, descriptive model which best describes the overall effect of P loading on the overall wetland biogeochemical cycling of N within the *Typha*, mixed vegetative zone and the *Cladium* area. Chapter 7 reiterates the study objectives and includes summary and concluding remarks as to the completion of each objective.

CHAPTER 2
INFLUENCE OF PHOSPHORUS LOADING ON ORGANIC NITROGEN MINERALIZATION

Introduction

Organic N mineralization (ammonification) in wetland soils is an important process in regulating water column inorganic N concentrations and provides a steady N supply to aquatic vegetation for growth. Ammonification, or the net release of ammonium N (NH_4-N) is a continuous, decomposition process by which high molecular weight, organic N compounds are sequentially hydrolyzed into simpler compounds by extracellular enzyme activity, followed by the breakdown of dissolved amino acid compounds and release of NH_4-N (Fuhrman and Bell, 1985; Gardner et al., 1989). The rate limiting step can occur anywhere along the decomposition continuum, but the process is generally limited by the rate of hydrolysis of the larger organic compounds (Stanford and Smith, 1972).

Ammonification is dependent on a number of biophysiochemical factors including the C:N ratio of the soil organic matter (SOM) and detrital tissue (Amador and Jones, 1997), temperature (Addiscott, 1983; Nyhan, 1976), O_2 status (Waksman and Purvis, 1932; Gale and Gilmour, 1988; Humphrey and Pluth, 1996; Amador and Jones, 1997), size and activity of the microbial pool (Amador and Jones, 1993; Wardle, 1992; Perucci, 1990), and limiting nutrients (Damman, 1988; Hossain et al., 1995; Munevar and

Wollum, 1977; Nair, 1996). Typically, organic matter additions to soil, assuming an average microbial C:N ratio of eight, exhibiting a C:N ratio > 25 will lead to N immobilization under aerobic conditions as the microbial biomass scavenge N during decomposition (Isirimah and Keeney, 1973). Net N mineralization has been observed to occur in flooded peat soils with C:N ratios of > 24:1 (Williams and Sparling, 1988), 45:1 (Humphrey and Pluth, 1996), and 80-100:1 (Damman, 1988). Therefore, there is little evidence that a specific C:N ratio can be applied to SOM in peat with general applicability for prediction of anaerobic organic N mineralization rates (Williams, 1984).

The microbial biomass sequesters N in organic forms (proteins, amino acids) which are released upon cell death. Inorganic N, released from the organic N pool, accumulates in wetland soils as NH_4^+ in lieu of undergoing transformation to NO_3^- due to the anaerobic status of the organic soil system (Reddy and Patrick, 1984) and diffusion limitations (Reddy et al., 1980). Increased soil moisture content of peat restricts the supply of O_2, leading to decreased organic matter decomposition rates (Humphrey and Pluth, 1996, Amador and Jones, 1997).

The availability of inorganic N is mediated by microbial activity. Microbial biomass has been significantly correlated with N mineralization rates (Franzleubbers et al., 1996, Williams and Sparling, 1988). The size and activity of the microbial pool, and therefore N mineralization, can be regulated by the availability of nutrients. It is well established that the size of the soil microbial biomass is dependent upon the C content of soils and additions of readily hydrolysable C sources results in increased microbial growth and activity (Anderson and Domsch, 1985; Schnurer et al., 1985). However, relationships between microbial biomass and soil organic carbon (SOC) have been shown to be strongest in soils with less than 2.5 % organic C (Anderson and Domsch, 1989; Wardle, 1992) and might not be applicable to high organic matter soils (Histosols).

Stimulatory responses to P additions on either the size or activity (represented by C or N mineralization) of the microbial biomass have been reported (Munevar and Wollum, 1977; Biederbeck et al, 1984; Prescott et al., 1992; Amador and Jones, 1993; Hossain et al., 1995; DeBusk and Reddy, 1998) while others have shown no response to P additions (Tate et al., 1991; Ross et al., 1995). Problems exist in assessing the effects of added P in upland agricultural sites including effects due to the simultaneous additions of N and P as well as extensive soil fertilization histories which can mask the overall effect of current P addition rates (Wardle, 1992). All the aforementioned factors, in concert with field scale soil heterogeneity, can make bulk soil net N mineralization estimates difficult.

Study Area

The Florida Everglades are currently affected by nutrient loading from urban and agricultural surface runoff. Most notably, this impact is seen in the Water Conservation Areas, one of the major hydrologic units of the Everglades (DeBusk et al, 1994). Water Conservation Area 2A has been receiving nutrient-laden (N and P) drainage waters for the past 40 years. Peat and nutrient (organic C, N and P) accretion rates have increased in areas receiving surface drainage water (Koch and Reddy, 1992; Craft and Richardson 1993). Most notably, the impact of anthropogenic nutrient loading is documented in the spatial distribution of surface soil total P. Total P concentration grades from a high of ~1600 mg kg^{-1} at the surface water inflow points to a background concentration of ~400 mg kg^{-1} in unimpacted areas, located in the interior of the marsh have been reported (Koch and Reddy, 1992; Reddy et al., 1993; DeBusk et al., 1994). A gradient in N and P of water column and periphyton tissue has also been documented along the same eutrophic transect in WCA-2A (McCormick and O'Dell, 1996).

Historically an oligotrophic, P-limited sawgrass (*Cladium jamaicense* Crantz) marsh, the vegetation began a shift towards a dominant cattail (*Typha domingensis* Pers.) vegetative community proximal to all surface water inflow points (Davis, 1991; Craft and Richardson, 1997). The timing of vegetative replacement coincided with the initiation of management of surface water pumped primarily from the canal network draining the Everglades Agricultural Area (EAA) approximately 40 years before present.

Objectives

The objectives of this study were to determine; (1) the potential ammonification rates of detritus and soil organic N under laboratory and field conditions; (2) the relationship between soil characteristics and short-term mineralization rates under drained and flooded soil conditions; and (3) the effect of added P on the size and activity of the soil microbial biomass and potential ammonification rates in P limited wetland soil.

Materials and Methods

Experimental Design

Eight stations were located along a 10 km transect originating from the S-10C inflow water control structure. The study transect spanned the marsh from a primary water control inflow structure (S-10C), southward across the dominant cattail (*Typha* sp.) vegetation and terminated approximately 10 km into the natural (unimpacted) marsh characterized by stunted stands of sawgrass (*Cladium* sp.) separated by shallow sloughs

dominated by floating and attached cyanobacterial mats. Sampling stations were located at distances of 1.4, 2.3, 3.3, 4.2, 5.1, 7.0, 8.4 and 10.1 km. Water depths varied seasonally from <2 cm to ~ 2 m along the transect length.

Sampling along the transect was not designed to identify differences between individual stations, but rather to investigate the gradient or trends in soil characteristics, including organic N mineralization, with distance. Soils were collected three times over a 1 yr period (February and August 1996, and March 1997). Sampling events were selected to best determine the effect of hydraulic loading rates of surface waters on ammonification rates, to include both the wet season (summer) and the dry season (winter).

The South Florida Water Management District (SFWMD) established 21 circular tanks enclosing 1.8 m^2 each and 3 open control plots in an unimpacted sawgrass-periphyton-slough. The mesocosm site was located approximately 11 km SW from the S-10C inflow water control structure (Figure 2-1). The dosing tanks were installed entirely within a shallow slough that contained no stands of sawgrass within the study site proper. The soil surface was dominated by floating and benthic cyanobacterial (periphyton) mats, purple bladderwort (*Utricularia purpurea* Walt.) and water lily (*Nymphaea odorata* Ait.) (McCormick and O'Dell, 1996). Three replicate tanks were selected at random and spiked with various amounts of NaH_2PO_4 mixed with slough water to achieve loading rates of 0, 0.4, 0.8, 1.6, 3.2, 6.4 and 12.8 g P m^{-2} y^{-1}. The tanks were closed for 24 h after spiking, then subsequently opened to permit exchange with the surrounding water. Prior to our soil sampling, these systems were dosed with P at respective levels starting in June 1995 and continued weekly for a period of 17 months.

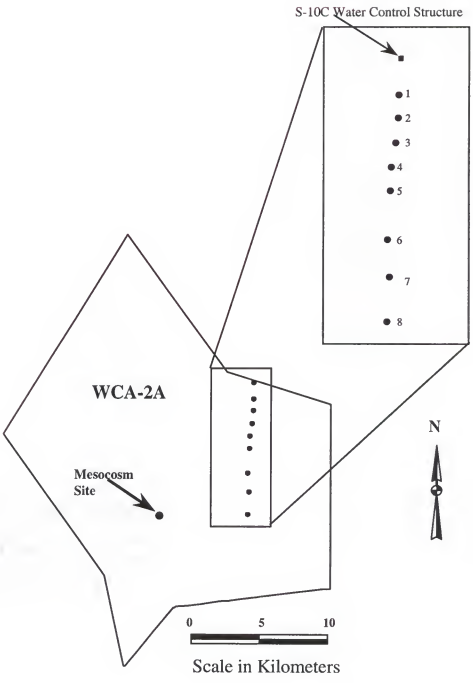

Figure 2-1. Station locations along soil phosphorous gradient in WCA-2A (south of S-10C water control structure) used in the study.

Soil Sampling

A minimum of four soil cores were collected within 5 m of each station by driving a 10 cm diameter aluminum irrigation pipe into the soil. A meter stick was used to measure the soil surface inside and outside the core to verify that negligible (<5%) compaction had occurred during coring. Cores were sealed, removed from the ground, immediately extruded and separated into separate soil intervals (0-10 and 10-30 cm) in the field. Each interval was well mixed to yield a representative and homogenous sample from each station. The August 1995 and February 1996 samples were bagged and immediately transported to the laboratory on ice. Samples were transferred into 2 L polyethylene containers within 24 h of collection and stored refrigerated at 4°C until analysis. Soil samples collected in March 1997 were immediately transported to a field "laboratory" location and incubated within 3 h of collection. The detrital material was collected during the last two sampling events for use in field incubations. Detritus consisted of recognizable, loosely associated cattail or sawgrass plant material lying on the surface of the more compact, brown, peat soil. The detrital layer varied in thickness from <1 cm in the sawgrass areas to >25 cm at the cattail stations closest to the inflow. The remaining soil samples not utilized in field incubations, were sealed in plastic bags and kept on ice until return to the laboratory where the samples were transferred into polyethylene containers and refrigerated at 4°C until subsequent characterization.

In order to investigate spatial field variability of soil characteristics and microbial processes, three stations at 2.3, 7.0, and 10.1 km from the inflow were sampled for detritus and 0-10 cm soil along a short, east-west transect, normal to the direction of the major sampling transect on October 13, 1997. Five individual cores were taken at 10 m intervals, sectioned in the field, stored in plastic bags and placed on ice until return to the

laboratory the following day for characterization of soil and potential net N mineralization determinations.

Soils were collected on November 21, 1996 from each of the mesocosms by driving a 10 cm diameter polyethylene tube into the soil. The periphyton-floc layer was poured off into separate sampling containers. The top soil interval (0-3 cm) was then extruded, stored in plastic bags and placed on ice until returning to the laboratory where samples were stored refrigerated at 4°C until subsequent characterization.

Soil Characterization

Bulk density was calculated for the soil intervals on a dry weight basis. Bulk density was not determined for detritus. Total C and N content of detritus and soils was determined on dried, ground samples using a Carlo-Erba NA-1500 CNS Analyzer (Haak-Buchler Instruments, Saddlebrook, NJ). Total P analysis was performed on sub-samples prepared by nitric-perchloric acid digestion (Kuo, S. 1996). Total P was determined using an automated ascorbic acid method (Method 365.4, USEPA, 1983).

Extractable NH_4-N was determined by shaking triplicate soil samples with 25 ml of 2 *M* KCL at a ratio of approximately 1:40 (g dry soil:extractant) for 1 h on a longitudinal shaker. Samples were centrifuged for 10 min and vacuum-filtered through Whatman #42 filter paper. The supernatant was collected and refrigerated at 4°C and determined colorimetrically for NH_4-N (EPA method 351.2, 1983).

Microbial Biomass

Microbial biomass C (MBC) was determined by the chloroform fumigation-extraction (CFE) technique after Vance et al. (1987). Six replicate 5 g samples were placed into 25 ml centrifuge tubes for each soil interval and all eight sampling sites. One half ml of chloroform was added to three replicate tubes and placed into a vacuum desiccator with a beaker containing 300 ml of chloroform and several boiling chips. The air was evacuated three times until the chloroform began boiling in the samples and beaker (~ 2 min). Each time, air was allowed back into the desiccator by means of a screw control valve on the lid. After the third evacuation, the desiccator was sealed under pressure for 24 h as the chloroform filled the headspace, making contact with the soil. The control set was placed on the adjacent lab bench. After 24 h, the headspace air was alternatively evacuated and filled with room air nine times to remove any presence of chloroform still present in the soil or beaker. Samples were removed and both the controls (not exposed to chloroform) and chloroform treated soils were immediately extracted with 20 ml of 0.5 *M* K_2SO_4, agitated for 30 min on a longitudinal shaker and vacuum filtered through #42 Whatman filter paper. The supernatant was collected and refrigerated at 4°C until analyzed on a Dohrman TOC analyzer. Microbial biomass carbon was determined by subtracting the extractable total organic carbon (TOC) in the triplicate controls from the triplicate chloroform-treated samples. An extraction efficiency (k_{EC}) factor of 0.37 was applied, utilizing the previous calibration for organic soils by Sparling et al. (1990). The values of TOC for the unfumigated (control) samples are defined as extractable C or labile C.

Microbial biomass N (MBN) was determined by the CFE technique after Brookes et al. (1985). Ten ml of extract from the microbial carbon procedure was subjected to

Kjeldahl-N digestion using the salicylic acid modification of Bremner and Mulvaney (1982). Samples were brought to a total volume of 20 ml after digestion, agitated on a vortex shaker and transferred into 30 ml scintillation vials. Extracts were analyzed for NH_4-N colorometrically (EPA Method 351.2, 1983). Microbial biomass N was determined by subtracting the extractable NH_4-N of the triplicate non-fumigated samples from triplicate fumigated samples. A combined extraction efficiency and k_{EN} value of 0.54 was applied (Brookes et al., 1985).

Potentially mineralizable nitrogen (PMN)

Potentially mineralizable N (PMN) was determined from anaerobic incubations after the method of Waring and Bremner (1964). There are four distinct advantages to using the PMN method over aerobic methods for studies of wetland soils; (1) only NH_4^+ needs to be measured, (2) higher temperatures, resulting in faster N release and shorter incubation times, can be used as optimal temperature for nitrification need not be maintained, (3) the anaerobic incubation environment is more applicable to field conditions at our study site, and (4) there is a prevention of moisture loss during the incubation. These factors combine to provide a reliable, relatively quick and less time intensive method for investigating differences in potential ammonification rates of wetland soils.

The PMN rate of soils and detritus were determined for the February 1996, August 1996, March 1997, and the spatial study samples (October 1997). Glass serum bottles were prepared by adding ~ 10 g of moist soil and 5 ml of distilled, de-ionized water. Bottles were capped with butyl rubber stoppers and sealed with aluminum crimps.

The headspace was evacuated and replaced with 99.99% O_2-free N_2 gas. Triplicate serum bottles were incubated in the dark at 40°C for 10 d. Selected serum bottles were monitored over the incubation to insure continuous anaerobic conditions by withdrawing 100 ul of headspace gas and determining the O_2 content by gas chromatography. Samples were removed from the incubator at the terminus of the incubation and extracted with 30 ml of 2 *M* KCL. Bottles were shaken for 1 h on a longitudinal shaker and centrifuged for 10 min at 6000 rpm. The supernatent was filtered through Whatman #42 filter paper, collected in 25 ml scintillation vials and refrigerated at 4°C for subsequent automated, colorometric analysis (EPA Method 351.2, 1983). Bottle incubations have routinely been used to investigate net N mineralization rates of detritus and soil (Waring and Bremner, 1964; Satathchandra, et al., 1989, Joegensen et al., 1990; Perucci, 1990; and Gale et al., 1992).

Substrate induced organic nitrogen mineralization (SINM)

Previous studies have found that NH_4-N can be immobilized during aerobic laboratory incubations at temperature < 35°C, thereby exhibiting no initial net mineralization (Gale et al., 1992; Ross et al., 1995; Trehan, 1996; Franzluebbers, 1996). Differences in net N mineralization can be investigated over short time periods (h) with simple substrates (amino acids) because the rate limiting steps of SOM breakdown and decomposition have been removed by providing a readily hydrolyzable substrate. The C:N ratio of various amino acids is much lower than the C:N ratio of the microbial pool resulting in substantial net N release. The concomitant release of CO_2 and NH_4^+ allows the investigator an option as to which analyte to measure. In this fashion, amino acid

utilization as a respiratory substrate (electron donor) provides a measure of the activity of the heterotrophic microbial population (Alef et al., 1988, Hopkins et al, 1994; Hopkins et al, 1997).

The substrate induced N mineralization (SINM) method has several advantages over other methods of assessing the size and activity of the microbial pool. First, the method can be easily applied to a large number of samples with incubation times of only a few hours. Alternatively, chloroform fumigation incubation (CFI) requires a 10 d incubation and both CFI and CFE (generally a 24 h incubation) require special precautions to handle vapors from the fumigant. Additionally, SINM can easily be applied in the field, allowing use of freshly collected soils to determine microbial activity preventing changes in the microbial metabolic status due to storage, refrigeration or lengthy incubation periods. Substrate induced N mineralization has demonstrated remarkable linearity over the short term (a few hours) and has been shown to occur in soils with minimal or no lag phase. This suggests this method measures the presently active soil microbial population responsible for N mineralization without affecting sufficient time for significant turnover and new biomass production (Alef and Kleiner, 1986; Hopkins et al., 1995; Franzluebbers et al., 1996).

Soil and detritus were collected from each of two stations per day over four days for a total of eight stations in August 1996 and March 1997 along the study transect. After thorough mixing, large (~100 g) subsamples were taken and split into two groups; one set for incubation under drained conditions and the other for flooded conditions. Moisture was removed from the drained samples by spreading thin layers of sample on dry sponges covered by sheets of Whatman glass fiber filter paper for 20-30 min. Soils utilized in the anaerobic incubations remained in field saturated condition (flooded).

Approximately 10 g of drained soil per station and depth increment were added to 250 ml Nalgene polyethylene bottles for aerobic incubations. Ten g of field moist soil were added to 250 ml centrifuge tubes for anaerobic incubation, equipped with a rubber septa embedded in the cap to provide a gas purging port. To triplicate samples of drained and flooded samples, 0.5 ml of solution containing 200 mg *L*-alanine ($C_3H_7NO_2$)-N L^{-1} were added by mechanical pipette. Samples were well mixed to distribute the spike solution and to break up soil clods using a glass rod in order to maximize the soil volume in contact with air (drained samples). Drained sample bottles were capped, incubated in the dark and submerged in site water in coolers for 4 h at ambient temperature (~28-31°C). Flooded sample bottles were capped, purged with O_2-free (99.99% pure) N_2 gas for 5 min, incubated in the dark, and submerged in site water in coolers for 4 h at ambient temperature.

Triplicate controls were included for the drained and flooded treatments. All samples were extracted at the terminus of the incubation with 50 ml of 2 *M* KCL. Bottles were agitated on a longitudinal shaker for 1 h and vacuum filtered through #42 Whatman filter paper. The supernatant was collected and kept on ice until returning to the laboratory where they were stored refrigerated at 4°C until subsequent automated colorimetric analysis for NH_4-N (USEPA Method 351.2, 1983).

Samples collected at 3 stations along the transect in October 1997 were brought back to the laboratory and were subjected to the SINM procedure within 24 h to investigate the within-station, spatial variability of amino acid utilization by the microbial populations.

The substrate chosen for the SINM experiment was the *L* isomer of alanine. The *L* isomer was chosen because previous studies have documented the greater preference of microbial populations for the *L* form of amino acids instead of the naturally, less

abundant *D* isomer (Hopkins and Ferguson, 1994; Hopkins et al., 1997). This preference is likely due to the fact that all cellular proteins are composed of *L* form amino acid isomers, while the *D* isomers are found primarily as a minor constituent of the peptidoglycan in the bacterial cell wall. Another reason for the selection of alanine is related to its low C:N ratio. Microbial utilization of a low C:N substrate will require the release of inorganic N in order for the cells to maintain a steady state C:N composition and should, therefore, be an excellent indicator for heterotrophic potential even in these high NH_4^+ organic soils (Gardner et al., 1989).

Nutrient Addition Study

Surface soil (0-10 cm interval) from Station 10 (10.1 km from the inflow) was collected in order to determine the effect of added inorganic P on potential ammonification rates of organic N. The soil was homogenized by mechanical mixing after removing live roots. Samples were pre-incubated by placing approximately 50 g of field moist soil in 120 ml media bottles equipped with a butyl rubber septa embedded in the cap. To each bottle, 40 ml of distilled deionized water was added and mixed well with the soil. The following treatments were evaluated; (1) control-no additions and (2) PO_4-P added (0.1, 1.0, 5.0, 10 mg L^{-1} porewater concentration). Each treatment was performed in triplicate. Bottles were capped and purged with O_2-free N_2 gas to create anaerobic conditions. Samples were incubated in the dark at 30°C for 10 d and were shaken by hand for 30 sec d^{-1}. Bottles were then re-spiked with the same concentration of respective solutions and incubated for an additional 10 d. Triplicate soil controls were spiked with distilled de-ionized water and included in the incubation. At the terminus of the 20 d incubation, 20 ml of slurry was collected from each bottle by pipette, and

extracted with 2 *M* KCL to determine extractable NH_4^+. An additional 10 ml were placed in serum bottles, under a O_2-free N_2 headspace for incubation at 40°C for 10 d. Samples were extracted with 2 *M* KCL at the terminus of the incubation and analyzed for extractable NH_4^+ for anaerobic PMN determinations.

Data Analysis

Soil characteristics and parameters were statistically related using Pearson's product-moment correlation and regression. Data were fitted to an ANOVA model to investigate significant differences ($P < 0.05$) in soil characteristics and ammonification rates among soil intervals (depth), distance from inflow, and nutrient addition levels (laboratory and field). Fisher's Least Significant Difference (LSD) test was utilized to determine significant difference between treatments for the nutrient addition study using the StatGraphics software program (Manugistics, Inc., Rockville, MD).

Results and Discussion

Soil Characterization

The organic soils contained high weight percent moisture contents (90-95 %) and low dry weight bulk densities averaging 0.064 (S.E.=0.0023) and 0.089 (S.E.=0.0029) g cm^{-3} for the 0-10 cm and 10-30 cm soil depths, respectively with significantly ($P < 0.001$) higher bulk densities in the underlying soil depth (Table 2-1). Significant increases in bulk density of peat with depth have been reported for 14 ombrotrophic peat bogs in Newfoundland, New Zealand, Nova Scotia and the northern United States (Damman,

Table 2-1. Select physiochemical properties of detritus and soils collected from along the transect in WCA-2A. Data are mean values (n=3) with 1 standard deviation in parentheses. Samples were collected February and August, 1996 and March 1997.

Distance	Depth	Bulk Density	Total C	Total N	Total P
km		$g\ cm^{-3}$	---------- $mg\ g^{-1}$ ----------		$mg\ kg^{-1}$
1.4	detritus	n.d.	450 (24.1)	25.9 (2.8)	1601 (287)
2.3	detritus	n.d.	432 (10.4)	25.8 (1.4)	1615 (177)
3.3	detritus	n.d.	443 (20.2)	22.0 (3.6)	1509 (122)
4.2	detritus	n.d.	431 (19.5)	21.0 (3.1)	1456 (459)
5.1	detritus	n.d.	434. (16.6)	20.7 (2.0)	1096 (274)
7.0	detritus	n.d.	414 (47.8)	21.8 (4.8)	829 (256)
8.4	detritus	n.d.	403 (24.0)	24.8 (4.2)	556 (177)
10.1	detritus	n.d.	419. (7.6)	22.9 (3.5)	417 (25)
1.4	0-10 cm	0.049 (0.006)	400 (20)	27.0 (2.3)	1552 (102)
2.3	0-10 cm	0.050 (0.016)	440 (14)	28.2 (2.5)	1369 (100)
3.3	0-10 cm	0.052 (0.011)	455 (33)	29.6 (4.1)	1205 (105)
4.2	0-10 cm	0.070 (0.015)	449 (40)	29.0 (3.4)	989 (144)
5.1	0-10 cm	0.067 (0.011)	433 (15)	27.4 (3.0)	966 (61)
7.0	0-10 cm	0.060 (0.006)	424 (34)	30.0 (2.2)	641 (152)
8.4	0-10 cm	0.067 (0.005)	445 (31)	29.3 (2.2)	486 (113)
10.1	0-10 cm	0.060 (0.002)	440 (23)	28.4 (4.0)	479 (124)
1.4	10-30 cm	0.086 (0.018)	444 (5.6)	32.0 (0.4)	570 (98)
2.3	10-30 cm	0.104 (0.011)	464 (9.8)	30.1 (2.7)	373 (10)
3.3	10-30 cm	0.094 (0.003)	474 (27)	33.8 (5.5)	363 (68)
4.2	10-30 cm	0.092 (0.006)	469 (31)	31.8 (4.9)	287 (38)
5.1	10-30 cm	0.074 (0.012)	395 (113)	26.4 (5.6)	289 (31)
7.0	10-30 cm	0.098 (0.004)	427 (35)	25.2 (4.0)	247 (84)
8.4	10-30 cm	0.088 (0.001)	478 (26)	28.6 (3.9)	229 (14)
10.1	10-30 cm	0.074 (0.009)	474 (21)	30.2 (3.9)	246 (32)

n.d. = not determined

1988). Bulk density was not determined for detritus. Total C and N values did not vary significantly along the transect and values were similar to those found in previous studies in WCA-2A (Koch and Reddy, 1992; DeBusk et al., 1994). Total C and N were significantly correlated with depth ($P < 0.01$). The increase of total C and N with depth might be tied to vegetative changes of which the peat was composed. Total C was significantly correlated with total N ($P < 0.01$; $r = 0.49$) for all samplings of detritus and soil along the transect. The mean C:N ratio was 16:6 (S.E. = 0.34) and failed to demonstrate a significant correlation with distance from the inflow.

Total P was significantly negatively correlated ($P < 0.01$; $r = -0.59$) with distance from the inflow for all samples (Table 2-2). Total P for detritus, 0-10, and 10-30 cm soil intervals taken separately were significantly negatively correlated with distance ($r = -0.89$, -0.95 and –0.74, respectively; Figure 2-2). Total P was significantly correlated ($P < 0.01$) with depth ($r = 0.68$) and was significantly higher ($P < 0.05$) in both detritus and 0-10 cm soil when compared with the underlying 10-30 cm soil. There was no significant difference in total P content of detritus and the 0-10 cm soil interval. The N:P ratio of soil and detritus increased with distance from inflow for detritus and soil ($P < 0.01$; Table 2-2).

Extractable NH_4^+ was significantly ($P < 0.01$) negatively correlated with depth averaging 377, 122 , and 55 mg N kg^{-1} for the detrital, 0-10 and 10-30 cm soil depth, respectively. Decreasing concentrations of extractable NH_4^+ in soils with increasing depth have been noted by others (Humphrey and Pluth, 1996). There was a highly significant correlation of extractable NH_4^+ with total P suggesting a possible link between inorganic N availability and total P along the transect (Table 2-2).

Bulk density did not vary significantly between treatments ($P > 0.9$) and averaged 0.092 g cm^3 for the 0-3 cm soil interval (Table 2-3). Total C and N values did not vary

Table 2-2. Product moment correlation coefficients calculated for soil characteristics and processes measured on detritus, 0-10 cm, and 10-30 cm soil layer along the transect in WCA-2A for February 1996, August 1996, and March 1997. For n=72, r =0.232 is significant at $P < 0.05$ and r = 0.302 is significant at $P < 0.01$. (TC=total carbon; TN=total nitrogen; TP=total P; Extr NH_4^+=extractable NH_4-N; MBC=microbial biomass carbon; MBN=microbial biomass nitrogen; PMN=potentially mineralizable nitrogen).

	Distance	TC	TN	TP	Extr NH_4^+	MBC	MBN	PMN	Depth	C:N ratio
TC	-0.05									
TN	-0.11	0.49								
TP	-0.59	-0.31	-0.29							
Extr NH_4^+	-0.15	-0.11	-0.53	0.47						
MBC	-0.25	-0.30	-0.53	0.70	0.73					
MBN	-0.19	-0.30	-0.56	0.61	0.81	0.86				
PMN	-0.28	-0.29	-0.48	0.65	0.78	0.81	0.85			
Depth	0.00	0.46	0.56	-0.68	-0.63	-0.69	-0.67	-0.59		
C:N ratio	0.08	-0.05	-0.87	0.16	0.58	0.47	0.50	0.41	-0.41	
N:P ratio	0.43	0.45	0.45	-0.86	-0.54	-0.68	-0.62	-0.58	0.86	-0.30

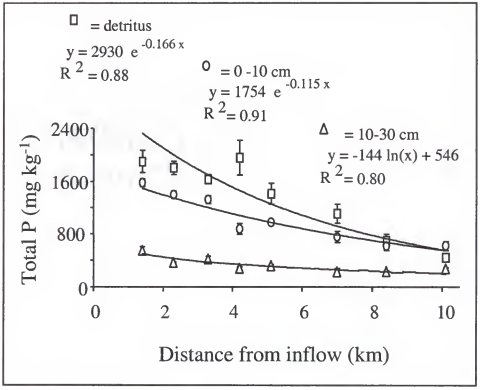

Figure 2-2. Total P vs distance from inflow for detritus, 0-10 cm and 10-30 cm soil depth intervals from along the transect in WCA-2A for the February 1996, August 1996 and March 1997 sampling dates. Plotted are means (n=3) and one standard error.

Table 2-3. Selected physiochemical properties of the 0-3 cm soil interval from the mesocosm experiment in WCA-2A. Data are mean values (n = 3) with 1 standard deviation in parentheses. Samples were collected October 1997.

P-loading rate	Bulk Density	Total C	Total N	Total P
g P m^{-2} y^{-1}	g cm^{-3}	-------- mg g^{-1} --------		mg kg^{-1}
0 (oc)	0.081 (0.008)	392 (9.3)	30.2 (3.1)	492 (56)
0	0.094 (0.024)	404 (25)	31.8 (4.6)	427 (41)
0.4	0.113 (0.023)	345 (65)	26.6 (6.5)	475 (65)
0.8	0.101 (0.032)	349 (11)	26.4 (2.0)	471 (71)
1.6	0.074 (0.015)	424 (4.2)	32.9 (0.5)	380 (55)
3.2	0.096 (0.033)	366 (48)	25.4 (2.2)	460 (34)
6.4	0.093 (0.011)	383 (26)	29.5 (3.8)	594 (40)
12.8	0.093 (0.034)	355 (62)	27.6 (7.6)	688 (240)

(oc) - open control

significantly among treatments, but were significantly correlated with each other ($P < 0.01$; $r = 0.86$). The mean C:N ratio was 13.2 (S.E. = 0.22) for the 0-3 cm surface soil and was not significant among P treatments. Total P of soil was significantly correlated to the experimental P loading rate indicating that P was not completely scavenged by the benthic periphyton/floc layer (Table 2-4). A significant decrease in the N:P ratio of the 0-3 cm soil was observed with increased P loading, ranging from a mean 75:1 in the no dose treatment to 45:1 in the highest P dose treatment. Extractable NH_4^+ was significantly correlated P loading rate and soil total P, suggesting a link between these parameters for both the transect study and the P enrichment field study (Table 2-4).

Microbial Biomass

Microbial biomass C and N in detritus were significantly negatively correlated with distance ($r = -0.68$; -0.48. A weak significant correlation was observed for MBC and MBN vs total P ($r = 0.65$; 0.41) for the detrital layer suggesting that P-loading, nearest the inflow point, might have influenced the size of the microbial biomass due to a historical P limitation (Davis, 1991; Figure 2-3).

Microbial biomass C and N also decreased with depth along the transect ($P < 0.01$; $r = 0.69$; 0.67 respectively). MBC averaged 13.3, 4.8 and 1.6 g kg^{-1} and MBN averaged 1090, 347 and 109 mg kg^{-1} for detritus, 0-10 cm and 10-30 cm respectively (Figure 2-4). The decrease in MBC and MBN with depth is likely due to the reduced availability of nutrients, in particular carbon, to support microbial populations in the subsurface and is similar to results found in other studies (DeBusk, 1996; Franzluebbers et al., 1995; Hossain et al., 1995; Williams and Sparling, 1988). The lignin-cellulose

Table 2-4. Product moment correlation coefficients calculated for soil characteristic and processes measured on the 0-3 cm soil interval in the mesocosm sampling in WCA-2A. For n=24, r is significant at $P < 0.05$ at r = 0.404 and $P < 0.01$ at r = 0.515. (TC=total carbon; TN=total nitrogen; TP=total P; MBC=microbial biomass carbon; MBN=microbial biomass nitrogen; Extr NH_4^+=extractable NH_4-N;Extr C=extractable carbon; PMN=potentially mineralizable nitrogen).

	P-Loading	TC	TN	TP	MBC	MBN	Extr NH_4^+	Extr C
TC	-0.18							
TN	-0.14	0.86						
TP	0.69	-0.24	-0.31					
MBC	0.32	-0.36	-0.34	0.50				
MBN	0.50	-0.02	-0.07	0.70	0.78			
Extr NH_4^+	0.45	0.04	0.02	0.55	0.50	0.60		
Extr C	0.05	0.52	0.44	0.15	0.23	0.38	0.44	
PMN	0.29	-0.07	-0.10	0.55	0.45	0.43	0.62	0.44

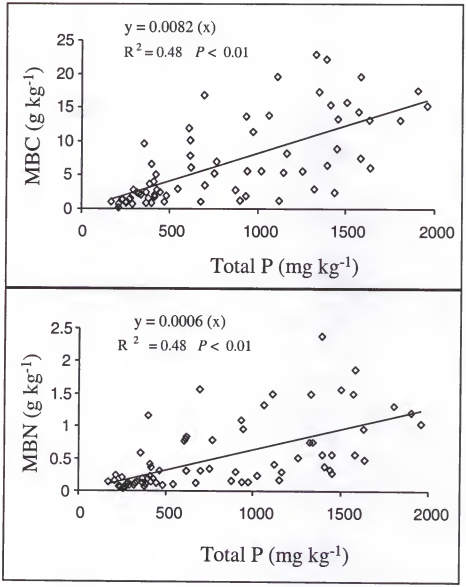

Figure 2-3. Microbial biomass C (MBC) and microbial biomass N (MBN) vs total P for detritus, 0-10 cm, and 10-30 cm soil intervals from along the transect in WCA-2A for the February 1996, August 1996, and March 1997sampling dates (n = 72).

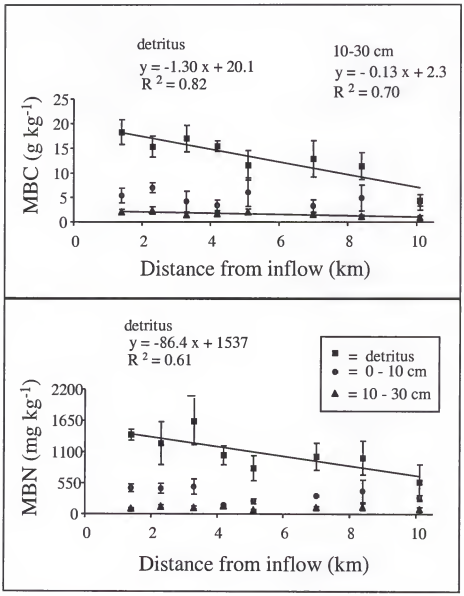

Figure 2-4. Microbial biomass carbon (MBC) and nitrogen (MBN) vs distance from inflow for detritus, 0-10 cm and 10-30 cm soil intervals along transect in WCA-2A. Plotted are mean values and one standard error for February 1996, August 1996 and March 1997 sampling dates.

index, a measure of relative C availability, was found to increase with depth indicating the relative abundance of the more recalcitrant substrate (lignin) which is less available to the heterotrophic microbial population (DeBusk and Reddy, 1998).

Another method of assessing the relative availability of C and N involves comparing the MBC and N pool sizes to soil total C and N (Anderson and Domsch, 1989). Microbial biomass C as a % of total C averaged 3.11 (S.E. = 0.27), 1.10 (S.E. = 0.15), and 0.36 (S.E. = 0.04) and MBN averaged 4.70 (S.E. = 0.52), 1.23 (S.E. = 0.14), and 0.37 (0.04) as a % of total N for detritus, 0-10 cm, and 10-30 cm depths, respectively. Values for C and N for both soil depths are within ranges reported by others (McLatchey and Reddy, 1998; Sparling and West, 1989; Inubushi et al., 1992) while values of the detrital layer are somewhat higher than reported. These results suggest that detritus is the most microbiologically active portion of the wetland soil profile and is likely to be responsible for the greatest amount of nutrient turnover/release.

A significant correlation was observed between MBC and MBN for the transect ($P < 0.01$; $r = 0.86$) averaging 12.3, 13.9 and 14.7 for detritus, 0-10 and 10-30 cm soil depth intervals, respectively. The apparent increase in C:N ratio of the biomass pool with depth might reflect an increasing limitation of inorganic N. The best-fit linear predicted an average C:N ratio of 11.4 for the microbial biomass.

Microbial biomass N was weakly correlated with P-loading rate ($r = 0.50$) for the 0-3 cm soil interval. The MBN were more strongly correlated with soil total P ($r = 0.70$) providing evidence that P was likely the limiting nutrient to the microbial biomass in natural Everglades peat soils.

The mean MBC was 7.1 g kg^{-1} while MBN averaged 519 mg N kg^{-1}. A strong correlation between MBC and MBN was observed ($P < 0.01$; $r = 0.78$) yielding an average C:N ratio of 9.9 for the microbial pool (Figure 2-5a). The similarity in average C:N ratio of microbial biomass pools from the transect and mesocosm studies does not address the possibility of differences in the relative, functional, microbial pool composition (Drake et al, 1996). The average ratios of MBC to total C and MBN to total N were 2.0 (S.E. = 0.20) and 1.8 % (S.E. = 0.34), respectively. These values were higher than the values for the 0-10 cm depth but lower than the detrital layer from along the transect.

The best fit regression of MBC vs MBN was linear but did not pass through the origin. The positive y-intercept of the regression equation signified an increasing C:N ratio with decreasing MBN. This distribution suggests a decreasing C:N ratio with increasing P loading (or increased MBN). The C:N ratio of the microbial biomass was plotted vs MBN in order to investigate the effects of added P on inorganic N availability to the microbial pool (Figure 2-5b). A horizontal line through the data would suggest that P had no effect on inorganic N availability and the y-intercept would represent the average C:N ratio of the microbial biomass. However, there was an exponential increase in C:N ratio at decreasing MBN, suggesting an effect of P on inorganic N availability to the microbial biomass. Franzluebbers et al. (1995) also noted exponentially greater C:N ratios associated with lowest MBN values and have attributed this relationship to an increased inorganic N limitation at depth.

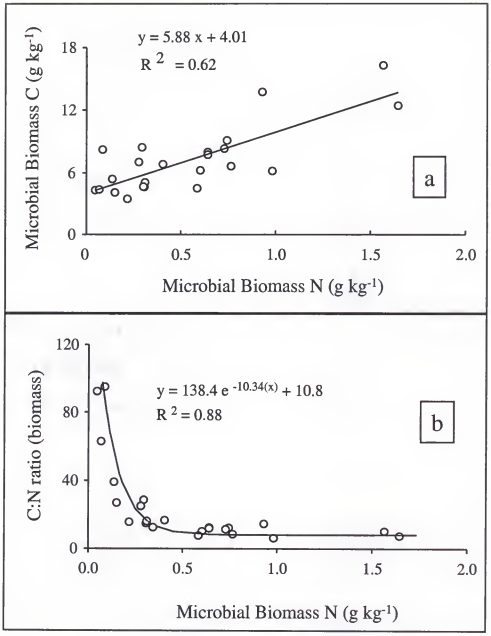

Figure 2-5. a) Microbial biomass C vs microbial biomass N, b) Microbial biomass C:N ratio vs microbial biomass N for the 0-3 cm soil from the mesocosm field study in WCA-2A.

Potentially Mineralizable Nitrogen

The potentially mineralizable nitrogen (PMN) rates were highest in the detrital layer, decreasing with depth averaging 126, 35.8, and 18.2 mg N kg^{-1} d^{-1} for detritus, 0-10 and 10-30 cm soil interval, respectively (Figure 2-6). A similar pattern of decreasing N availability with depth has been observed by others (Franzluebbers et al., 1996, Hossain et al, 1995). There existed a weak negative correlation of PMN rate with distance from inflow for all sample intervals combined ($P < 0.05$, $r = -0.28$), as well as for each depth interval taken separately with the most significant effect seen in detritus samples.

The results of the spatial study conducted in November, 1997 yielded PMN rates averaging 112 for detritus and 33.6 mg N kg^{-1} d^{-1} for the 0-10 cm soil interval. Microbial processes have been shown to be highly variable in the field with coefficient of variations (CV) on the order of 100 –200 % (Velthof, et al., 1996). The average CV for PMN rate of detritus and 0-10 cm samples was 28 %, which was higher than the variability observed on triplicate composite incubation samples. The CV for triplicate incubations averaged 7.8 %. This result support the statistical analyses of the data which treated the samples along the transect, not as individual sites (because like intervals from 4 cores taken at each station in the initial study were combined) but rather, as a distribution of soil characteristics along a gradient utilizing regression analysis.

The PMN rate was significantly correlated with several soil parameters including MBC, MBN, total P and extractable NH_4 (Table 2-2). These relationships might be useful in developing diagnostic biogeochemical indicators, however care should be taken to examine each relationship before proceeding from correlation to causation.

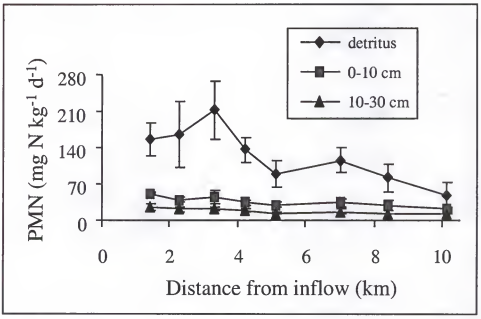

Figure 2-6. Potentially mineralizable N for detritus, 0-10 and 10-30 cm soil interval from along the study transect in WCA-2A for the February and August 1996 and March 1997 samplings. Plotted are means (n = 3) with one standard error.

The organic rich Everglades soils have a low redox potential and contain a thin (2-4 mm) oxidized layer due to high available C coupled with high microbial activity (Koch and Reddy, 1992; DeBusk, 1996). The low O_2 status of the soil can result in the near complete inhibition of the autolithotrophic conversion of NH_4^+ to NO_3^-. Therefore, the concentration of extractable NH_4^+ might provide a good indication of *in situ* ammonification rates in flooded soils (Ross et al., 1995; Williams and Sparling, 1988).

Additionally, our results support the use of the chloroform-fumigation extraction (CFE) method in wetland soils, criticized by some because it does not measure the active microbial pool but simply measures cellular products (primarily cytoplasm) of cells, whether they are active or not. Jenkinson (1988) stated that it is well to be clear about what biomass measurements do not provide. They are essentially standing crop measurements and not measurements of microbial activity such as mineralization of N. However, our findings suggest that for anaerobic incubations of high organic wetland soils, microbial biomass determined from CFE did an adequate job predicting potential ammonification rates, at least over the short term. The significant relationship of microbial biomass components to heterotrophic microbial activity demonstrates the influence the microbial biomass can exert on the concentration of extractable NH_4^+ in detritus and soil and therefore, the overall wetland water quality.

The mesocosm experiment provided an excellent opportunity for a separation of effects in the field, as P was loaded at several rates to soil at the same station containing similar vegetative characteristic and presumably, similar microbial populations. Unlike the transect study, where vegetation type and density as well as functional microbial communities vary (Drake et al, 1996), any differences in soil characteristic or microbial process should either be directly attributed to P enrichment.

Total P was significantly positively correlated with both MBC and MBN suggesting a P limitation to the microbial pool (Table 2-4). In addition, total P was significantly ($P < 0.01$) correlated with PMN rate indicating an increase in inorganic N release. Combining all the data from the transect and mesocosm studies, the best fit regression model of PMN and total P is exponential (Figure 2-7). Given the number of sources for variability over the transect length, the results suggest that differences in ammonification rates in WCA-2A were likely primarily controlled by availability of P to the microbial pool with 50% of the variability explained.

Extractable NH_4^+ is also significantly correlated ($P < 0.01$) with PMN rate for the mesocosm study. Combining all the data from the transect and mesocosm studies, there exists a significant relationship between PMN rate and extractable NH_4^+ with 59% of the variability in PMN explained by extractable NH_4^+ (Figure 2-8). This result supports the assertion that extractable NH_4^+ is a valid indicator of potential N mineralization and also suggests that the detritus is the least predictive component demonstrating the highest variability.

The relationship of PMN rate with both MBC and MBN is almost certainly one of causation (Figure 2-9). Ammonification is a microbial-mediated process and given a substrate (SOM) with a similar C:N ratio, one could expect differences in total active microbial biomass to influence the rate at which inorganic N is liberated from the organic fraction. These results suggest that total P is a reliable indicator of microbial activity in the soil , hence ammonification rates, due to the P limitation of the microbial pool in these soils. A similar P limitation to N mineralization was found for a volcanic

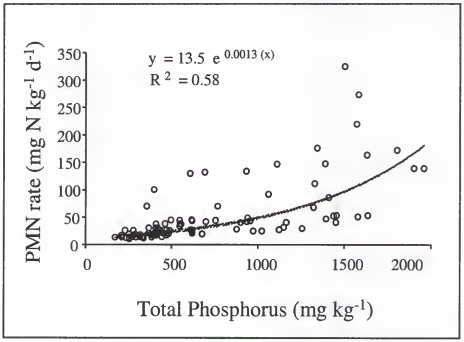

Figure 2-7. Potentially mineralizable N rate vs total phosphorus for all the mesocosm and transect samples combined.

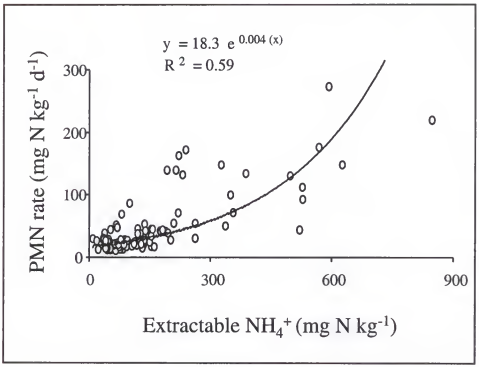

Figure 2-8. Potentially mineralizable N rate vs extractable NH_4^+ for all the mesocosm and transect samples combined.

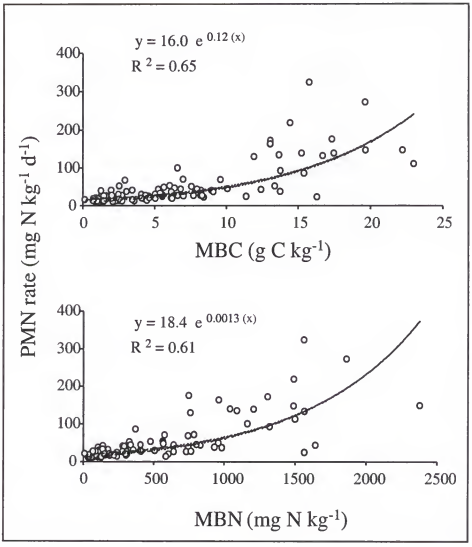

Figure 2-9. Potentially mineralizable N rate vs microbial biomass C and N for all the mesocosm and transect samples combined.

ash (Inceptisol) soil (Munevar and Wollum, 1977) and a peat soil from the Everglades National Park (Nair, 1996).

Nutrient Addition Study

Surface soil (0-10 cm) samples spiked and incubated with various levels of PO_4-P demonstrated a significant difference in PMN from the control over the entire range of P additions (Table 2-5). A similar result was found in bottle incubations under three levels of P loading for a peat soil from the Everglades National Park (Nair, 1996). There was no significant difference in microbial biomass among the treatments, however there was a significant increase in microbial biomass by CFE, during the length of the incubation. This result suggests that the increased availability of P added to the soil, increased the heterotrophic activity over the short term. The effect of continual P loading over longer terms (many months to years), seen in the results of the mesocsom and transect studies, not only resulted in increased microbial activity but an increased microbial pool size. Clearly, the increase in available P had a direct influence on increasing the specific heterotrophic microbial activity responsible for net N mineralization of native SOM.

Substrate induced N mineralization (SINM) field study

The SINM can be used to measure the relative activity of the portion of the microbial pool responsible for N mineralization in soil. Detritus and soil samples under drained conditions exhibited higher ammonification rates than samples under anaerobic,

Table 2-5. Potentially mineralizable nitrogen (PMN) rates for soil (0-10 cm) utilized in the nutrient addition study. Letters following rates depict significant differences (same letter = not significant).

Porewater Concentration	PMN rate
mg L^{-1}	mg N kg^{-1} d^{-1}
control	14.5 a
0.1 P	18.2 b
1.0 P	21.2 b
5.0 P	21.1 b
10.0 P	21.0 b

flooded condition with the highest rates found in the detrital layer. Under flooded conditions, the detrital layer, exhibited ammonification rates approximately two times greater than rates found in the 0-10 and 10-30 cm soil depths averaging 36.8, 22.8, and 21.7 mg N kg h^{-1} respectively. Mean ammonification rates of drained samples of detritus, 0-10 and 10-30 cm soil depths were 75.4, 51.0, and 43.1 mg N kg h^{-1}, respectively. On average, drained SINM rates were 1.99 (S.E. = 0.22) times greater than SINM rates under flooded conditions. The best fit linear regression between drained and flooded SINM rates yielded a slope of 2.14 (Figure 2-10). This suggests that organic N mineralization under drained conditions will proceed at a rate two times faster than flooded conditions.

The difference in rates between flooded and drained samples was due to the introduction of O_2 into the drained samples. The change in aeration status allowed facultative anaerobes to utilized O_2 as an electron acceptor leading to higher microbial rates. A similar difference in aerobic and anaerobic mineralization rates of alanine was reported for an organic soil from central Florida (McLatchey and Reddy, 1998) and a difference in aerobic – anaerobic C mineralization rates in an Everglades peat soil where aerobic rates were approximately three time greater than anaerobic rates (DeBusk and Reddy, 1998). Rates of alanine mineralization were one order of magnitude greater than those reported by McLatchey and Reddy (1998) and are likely a consequence of a larger active microbial pool present in our soils.

There existed no significant correlation for SINM vs depth. There was a significant ($P < 0.05$) very weak correlation with distance from inflow for both drained and flooded samples (r = -0.32; -0.38 respectively). A highly significant ($P < 0.001$)

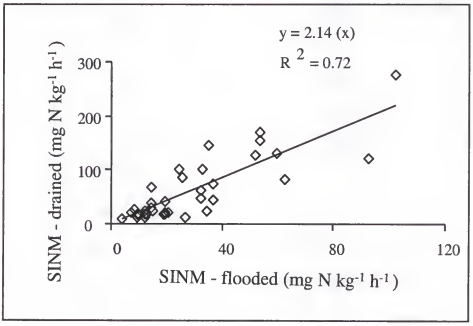

Figure 2-10. Substrate induced N mineralization rates for detritus and soil samples under drained and flooded conditions measured in the field from along the transect in WCA-2A. Relationship is significant ($P < 0.01$) for n = 42 samples.

seasonal effect was found with higher ammonification rates in the summer (August 1996) when compared to the winter (March, 1997). A portion of the difference in rates has to be attributed to differences in field incubation temperature (~ 6 C) between sampling dates, however differences in microbial activity are also likely responsible for the rate differences. Total C was positively significantly ($P < 0.01$) correlated and total N was significantly ($P < 0.05$) negatively correlated with SINM for drained and flooded samples (Table 2-6). Total P was not significantly correlated to either drained or flooded SINM.

Extractable NH_4^+ exhibited a weak correlation to SINM ($P < 0.01$; r = 0.53, 0.59) for both drained and flooded samples. The MBC and MBN were significantly correlated to SINM for the flooded samples. The size of the microbial pool, represented by MBN for the drained samples, was also significantly correlated with SINM. The release, or de-amination of N from amino acids has been seen by others in lake systems where N was not limiting (Gardner et al., 1987; Hollibaugh, 1978). The authors found increased NH_4^+ production (N mineralization) in samples fortified with amino acids was related to amino acid removal over the same time period. The significant relationship of microbial biomass and N mineralization rate conflicted with previous attempts to relate these same parameters for aerobic mineralization (Groot and Houba, 1995). The authors found neither MBC nor MBN were significantly related to N mineralization.

Result suggests that the microbial pool, with sufficient inorganic N available in the soil and therefore substantial NH_4^+ release when the amino acid was added, utilized the amino acid substrate primarily for carbon for energy. In order to utilize the carbon incorporated in the skeleton of the amino acid, enzymes must be produced, which further increases the net energy cost associated with the utilization of amino acids for C requirements (Wheeler and Kirchman, 1986). It is likely that despite the enormous pool

Table 2-6. Product moment correlation coefficients for substrate induced nitrogen mineralization (SINM) vs selected soil characteristics for the field study in WCA-2A. For n=42, r = 0.304 is significant at $P < 0.05$ and r = 0.393 is significant at $P < 0.01$ (TC=total carbon;TN=total nitrogen; TP=total phosphorus; Extr NH_4^+=extractable NH_4-N; MBC=microbial biomass carbon; MBN=microbial biomass nitrogen; PMN=potentially mineralizable nitrogen).

Parameter	Drained SINM	Flooded SINM
TC	0.42	0.39
TN	-0.33	-0.39
TP	0.14	0.25
Extr NH_4^+	0.53	0.59
MBC	0.23	0.34
MBN	0.30	0.38
PMN	0.08	0.19
Depth	0.21	0.25
Distance	-0.32	-0.38

of total C in these organic soils, little C is directly available to the microbial population (Amador and Jones, 1997; DeBusk and Reddy, 1998). In response, microbial populations tend to increase transamination and deamination through increased enzyme production in order to utilize the carbon skeleton of the amino acid for cellular function and growth.

Ecological Implications

An understanding of the complex association of the inter-related nutrient cycles is essential in fully understanding an ecosystem response to increased nutrients and is critical in preparing and managing a successful mitigation strategy to restore the Everglades system back to pre-impact plant community structure and function levels. The results of field studies from WCA-2A have shown P loading to the soil, over the time frame of months to 10's of years, has significantly increased soil total P. This obvious finding assists in ruling out alternative hypotheses for the total P distribution along the transect in WCA-2A (difference in total P due to vegetation type, distance effects, or differences in net accretion rates). In turn, elevated total P levels of detritus and soil have increased the size of the microbial pool in the historically P limited Everglades system and total P was therefore, a significant indicator of microbial pool size (Figure 2-11). Phosphorus enrichment has also led to increased soil accretion rates in WCA-2A (Reddy et al., 1993) and consequently provides a greater supply of organic substrate with which to feed the internal cycling of N.

The increased size of the heterotrophic microbial pool has caused a concomitant increase in release of inorganic nutrients including N, and essentially provides "enhanced fertilization" for macrophytes, which have flourished in the impacted area. The response

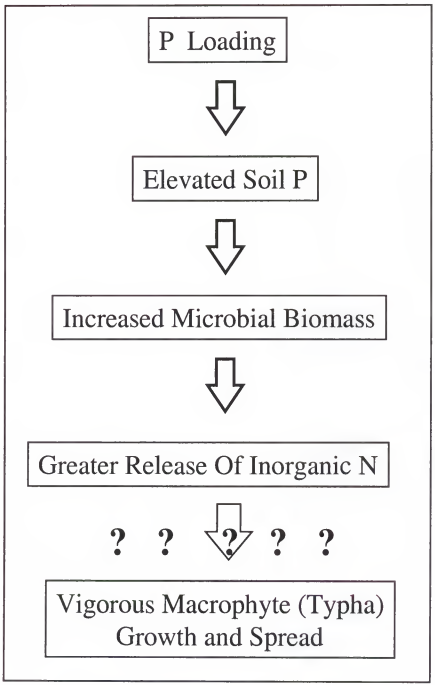

Figure 2-11. Diagram showing likely connections between P loading and the increased release of inorganic N with implications for vegetative replacement.

of *Typha domingensis* to the nutrient gradient has been found to be characteristic of species competitive in a fertile habitat while the response of *Cladium jamaicense* was found to mimic species in an infertile habitat (Davis, 1991). The fast growing *Typha* also demonstrated a competitive advantage, by allocating a majority of biomass into leaves in areas of enriched total P soil, essentially outcompeted the shorter, slower growing sawgrass for space, light, and perhaps available nutrients to form dense, monotypic stands in areas once dominated by *Cladium* (Miao and Sklar, 1998). The high density of *Typha* has essentially filled in natural open water areas once utilized by endemic and migratory bird populations. In this fashion, eutrophication has led to a shift in both ecosystem structure and function.

The effect of P enrichment on the biogeochemical cycling of N could have significant consequences for the water quality remediation strategy adopted by the SFWMD to prevent further eutrophication of the Northern Everglades. The current strategy consists of designing wetland treatment cells that significantly lower the concentration of P in water draining from the EAA before entering the WCAs. While it seems clear that P loading to the Everglades must be reduced, there exists significant a significant mass of P stored in the soils in the impacted region. The presence and continued release of P already stored in soils in impacted regions (Fisher, 1997) may continue to spread the zone of both P enrichment and elevated inorganic N release, which may further impact soil, vegetation and water quality of the northern Everglades. In addition, continued P recycling within the impacted region is likely to increase the biogeochemical turnover of N which has been suggested as a contributing mechanism to the *Typha* proliferation in WCA-2A.

Conclusions

Total P was significantly correlated with PMN and extractable NH_4^+ for the transect study and consequently provides an easily measurable biogeochemical indicator in investigating the impacts of eutrophication on ammonification rates. Total C and N proved to be ineffective biogeochemical indicators for prediction of microbial biomass or organic N mineralization. Total P proved to be a useful indicator for the relative pool size of MBC and MBN in the soil, as P loading significantly increased the microbial biomass of natural, P limited peat soil in WCA-2A. The size of the microbial pool in soil proved to be a reliable indicator of N mineralization potential. Results from the P dosing field study also suggest that a P limitation controlled the availability of inorganic N to the microbial pool, causing a decrease in the C:N ratio of the microbial biomass with increased P additions.

The results of the substrate addition field study suggest significantly higher SINM was due to a greater active microbial biomass. In addition, correlations of ammonification rates of *L* alanine with microbial biomass suggest that CFE was a reliable method for determining the active heterotrophic populations responsible for N mineralization. There was a significant difference in ammonification rates of native organic matter and *L* alanine, with the average PMN rate ~ 23 times slower than the SINM rates, correcting PMN rates for a Q_{10} of 2. Similar results have been seen for amino acid utilization in lake water (Gardner et al., 1989) and in soil samples (Alef and Kleiner, 1986). The fact that PMN and SINM were not correlated, as well as the large differences in ammonification rates lends additional support that N mineralization is

limited by the breakdown of the larger, more complex compounds while simple, amino acids compounds are quickly attacked by the microbes and utilized.

In short, eutrophication has increased the turnover rates of inorganic N from soil and detritus linked to an increased activity and size of the microbial pool. The microbial mediated mobilization of nutrients, through increased decomposition, has led to an increased availability of inorganic N, potentially impacting macrophyte growth, community structure and water quality of the northern Everglades system.

CHAPTER 3
INFLUENCE OF SELECTED INORGANIC ELECTRON ACCEPTORS ON ORGANIC NITROGEN MINERALIZATION

Introduction

Mineralization of organic forms of N is a key process regulating the bioavailability of N and consequently, the productivity of wetland ecosystems. Several key physiochemical factors influence the cycling of N in the soil-water column including the C:N ratio of soil organic matter (Amador and Jones, 1997), size and activity of the microbial biomass pool (Wardle, 1992; Perucci, 1990), temperature (Addiscott, 1983; Nyhan, 1976), and aeration status (Waksman and Purvis, 1932; Gale and Gilmour, 1988; Humphrey and Pluth, 1996). The mineralization of organic N in wetlands is carried out by a wide variety of functional, heterotrophic microorganisms. The microbial biomass is an essential regulator of nutrient availability and controls ecosystem function by metering the flow of energy to higher trophic levels in the decomposer food. Therefore, any factor that regulates the size and activity of the microbial pool, will also affect the biogeochemical cycling of N.

The redox status of the wetland soil system can exert substantial control over the cycling of N (Reddy and Patrick, 1975). Mineralization of organic N can proceed under both aerobic and anaerobic conditions. However due to the restricted supply of O_2, the influence of alternate electron acceptors on microbial catabolic processes can mediate the

rate at which organic matter decomposition occurs in wetland soils (D'Angelo and Reddy, 1999). The concept of free energy determines the order by which electron acceptors are utilized by the microbial populations; O_2, NO_3^-, SO_4^{-2}, and finally HCO_3^- reduction (McLatchey and Reddy, 1998). The microbial pool composition and rates of organic N mineralization will, therefore, vary under dominance of any single inorganic electron acceptor.

Microbial communities become established in the soil profile, dependent upon aeration status and the availability of inorganic electron acceptors. The aerobes would be found within the surface layer, NO_3^- reducers located further down, with the SO_4^{2-} reducers and methanogens located deepest in the soil profile, out of range of influence of O_2. This "layer cake" model of electron acceptor consumption has been demonstrated by Sorensen et al. (1979) for a coastal sediment. In addition, the authors noted that the aerobic, NO_3^- and SO_4^{2-} reduction layers were thin, with all maxima located within the top 1 cm of soil. In this fashion, an allochtonous supply of inorganic electron acceptors from surface water and ground water sources could significantly alter soil nutrient dynamics and wetland function.

Study Area

The Florida Everglades are currently affected by nutrient loading from urban and agricultural surface runoff. Most notably, this impact is seen in the Water Conservation Areas, one of the major hydrologic units of the Everglades (DeBusk et al, 1994). Water Conservation Area 2A has been receiving nutrient-laden (N and P) drainage waters for

the past 40 years. Peat and nutrient (organic C, N and P) accretion rates have increased in areas receiving surface drainage water (Koch and Reddy, 1992; Craft and Richardson 1993). Most notably, the impact of anthropogenic nutrient loading is documented in the spatial distribution of surface soil total P. Total P concentration grades from a high of ~1600 mg kg^{-1} at the surface water inflow points to a background concentration of ~400 mg kg^{-1} in unimpacted areas, located in the interior of the marsh, have been reported (Koch and Reddy, 1992; Reddy et al., 1993; DeBusk et al., 1994). A gradient in N and P of water column and periphyton tissue has also been documented along the same eutrophic transect in WCA-2A (McCormick and O'Dell, 1996).

Historically an oligotrophic, P-limited sawgrass (*Cladium jamaicense* Crantz) marsh, the vegetation began a shift towards a dominant cattail (*Typha domingensis* Pers.) vegetative community proximal to all surface water inflow points (Davis, 1991; Craft and Richardson, 1997). The timing of vegetative replacement coincided with the initiation of management of surface water pumped primarily from the canal network draining the Everglades Agricultural Area (EAA) approximately 40 years before present.

Objectives

The objectives of this study were to (1) determine rates of potential mineralization of organic N associated with detritus and soil under conditions of various inorganic electron acceptors dominance (aerobic, NO_3^-, and SO_4^{2-} reducing and methanogenic conditions), (2) determine rates of substrate induced mineralization of organic N, and

(3) investigate the relationship between easily measured soil characteristics (including total P) and N mineralization rates.

Materials and Methods

Experimental Design

Eight stations were located along a 10 km transect originating from the S-10C inflow water control structure (Figure 3-1). The study transect spanned the marsh from a primary water control inflow structure (S-10C), southward across the dominant cattail (*Typha sp.)* vegetation and terminated approximately 10 km into the natural (unimpacted) marsh characterized by stunted stands of sawgrass (*Cladium* sp.), separated by shallow sloughs, dominated by floating and attached cyanobacterial mats. Sampling stations were located at distances of 1.4, 2.3, 3.3, 4.2, 5.1, 7.0, 8.4 and 10.1 km. Water depths varied seasonally from <2 cm to ~ 2 m along the transect length. Sampling along the transect was not designed to identify differences between individual stations, but rather to investigate the gradient or trends between soil characteristics and selected N microbial processes, including aerobic and anaerobic organic N mineralization.

Soil Sampling

A minimum of four soil cores were collected within 5 m at each station by driving a 10 cm diameter aluminum irrigation pipe into the soil. A meter stick was used to measure the surface of the soil inside and outside the core prior to removal to verify that

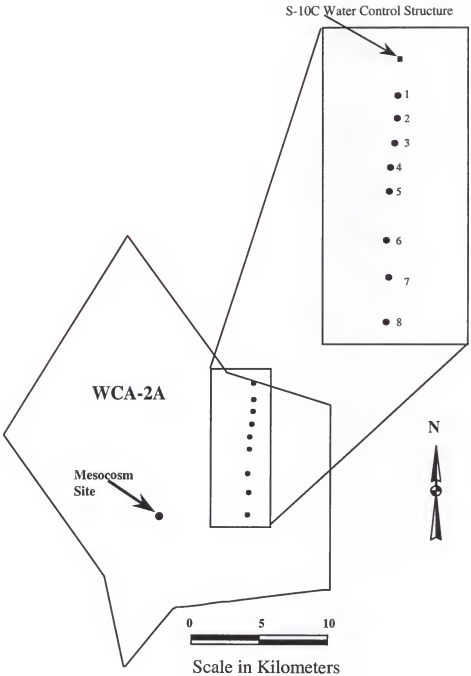

Figure 3-1. Station locations along soil phosphorous gradient in WCA-2A (south of S-10C water control structure) used in the study.

that negligible (<5%) compaction had occurred during coring. Cores were removed from the ground, immediately extruded, and separated into intervals (0-10 and 10-30 cm) in the field. Each sample interval was well mixed to yield a representative and homogenous sample from each station. Detrital plant litter was also collected at each station. Detritus consisted of recognizable, loosely associated cattail or sawgrass plant material lying on the surface of the more compact, brown, peat soil. The detritus layer varied in thickness from <1 cm in the sawgrass areas to >25 cm at the cattail stations closest to the inflow

Samples were transferred into 2 L polyethylene containers with 24 h of collection and stored refrigerated at 4°C until subsequent characterization. Samples of detritus and soil for the determination of anaerobic N mineralization rates were collected in March 1997 and samples for determination of aerobic N mineralization rates were collected in October 1997.

Soil Characterization

Moisture(% oven dry basis) was determined by drying ~ 20 g of a field moist sub-sample in a forced -air drying oven at 70°C. Bulk density was calculated for the soil intervals on a dry weight basis. Total C and N content of detritus and soils was determined on dried, ground samples using a Carlo-Erba NA-1500 CNS Analyzer (Haak-Buchler Instruments, Saddlebrook, NJ). Total P analysis was performed on sub-samples prepared by nitric-perchloric acid digestion (Kuo, S. 1996). Total P in the digestate was determined using an automated ascorbic acid method (Method 365.4, USEPA, 1983).

Soil oxygen demand (SOD) was determined for a single core collected in February 1996 at Station 1 (1.4 from inflow). The top 36 cm of soil were sectioned into 2 cm intervals and 10 g wet weight of sample were added to 300 ml of distilled, de-ionized water in a capped, continuously stirred BOD bottle. Dissolved O_2 was monitored using a YSI Model 58 oxygen meter with probe (Yellow Springs, CO). Soil oxygen demand was calculated as the difference in O_2 saturated soil:water slurry at t = 0 minus measured dissolved O_2 concentrations after 8 h, divided by the dry weight of the soil sample and time elapsed between measurements (APHA, 1992; 2-64,65).

Extractable NH_4^+ was determined by shaking triplicate soil samples with 25 ml of 2 *M* KCL at a ratio of approximately 1:40 (g dry soil:extractant) for 1 h on a longitudinal shaker. Samples were centrifuged for 10 min and vacuum-filtered through Whatman #42 filter paper. The supernatant was collected and refrigerated at 4°C and determined colorimetrically for NH_4-N (EPA method 351.2, 1983).

Microbial Biomass

Microbial biomass C (MBC) was determined by the fumigation-extraction technique after Vance et al. (1987). Six replicate 5 g samples were placed into 25 ml centrifuge tubes for each soil interval and sampling station. One half ml of chloroform was added to three replicate tubes and placed into a vacuum desiccator with a beaker containing 300 ml of chloroform and several boiling chips. The air was evacuated three times until the chloroform began boiling in the samples and beaker. Each time, air was allowed back into the desiccator by means of a screw control valve on the lid. After the third

evacuation, the desiccator was sealed under vacuum for 24 h as the chloroform filled the headspace, making contact with the soil. The control set was placed on the adjacent lab bench. After 24 h, samples were removed and both the controls (not exposed to chloroform) and chloroform treated soils were immediately extracted with 20 ml of 0.5 *M* K_2SO_4, agitated for 30 min on a longitudinal shaker and vacuum filtered through #42 Whatman filter paper. The supernatant was collected and refrigerated at 4°C. Dissolved organic C was determined on a Dohrman DC 190 carbon analyzer (Santa Clara, CA). Microbial biomass carbon was determined by subtracting the extractable total organic carbon (TOC) in the triplicate controls from the triplicate chloroform-treated samples. An extraction efficiency (k_{EC}) factor of 0.37 was applied, utilizing the previous calibration for organic soils by Sparling et al. (1990).

Microbial biomass N (MBN) was determined by the fumigation-extraction technique after Brookes et al. (1985). Ten ml of extract from the microbial carbon procedure was subjected to Kjeldahl-N digestion using the salicylic acid modification of Bremner and Mulvaney (1982). Samples were brought to a total volume of 20 ml after digestion and transferred into 30 ml scintillation vials. Extracts were analyzed for NH_4-N colorometrically (EPA Method 351.2, 1983). Microbial biomass N was determined by subtracting the extractable NH_4-N of the triplicate non-fumigated samples from triplicate fumigated samples. A combined extraction efficiency and Kn value of 0.54 was applied (Brookes et al., 1985).

Organic N mineralization under aerobic conditions

Aerobic N mineralization rates of detritus and soil were determined in constantly stirred reactors described by McLatchey and Reddy (1998). Approximately 300 g wet weight of soils and litter were placed in triplicate 1 L Erlenmeyer glass flasks and mixed with 400 ml of water. Flasks were placed on Thermolyne magnetic stirrers and equipped with floating, magnetic stir bars. Continual mixing, in concert with continuous aeration with room air, using an aquarium pump connected to glass tubing inserted through a butyl rubber stopper in each flask, helped maintain aerobic conditions in the slurry. The redox status of the soil slurries was monitored using a Fisherbrand Accumet 1002 combination electrode with platinum band (Fisher Scientific, Pittsburg, PA) and temperatures were recorded with mercury thermometers. The average temperature of the reactors was ~ 30°C. Reactors were wrapped with opaque paper to shield the samples from direct light.

Approximately 10 ml of slurry was collected from each reactor daily and extracted with 10 ml of 2 M KCL. Extracts were mixed well on a longitudinal shaker for 30 min, centrifuged for 10 min at 6000 rpm, and filtered through # 42 Whatman filter paper. The supernatant was refrigerated at 4°C for subsequent, automated, colorometric analysis of NH_4^+ (EPA Method 351.2, 1983) and NO_3^- (EPA Method 353.2, 1983).

Aerobic N mineralization rates were determined by summing the inorganic N at each sampling time ($NH_4^+ + NO_3^-$) and fitting a straight line through the data points. Rates were calculated by taking the slope of the best fit linear regression line through the data and were expressed as mg N kg dry weight soil $^{-1}$ d^{-1}.

Organic N mineralization under anaerobic conditions

The organic N mineralization rate of soils and detritus under dominant nitrate-reducing, sulfate-reducing and methanogenic conditions were determined for the March 1997 samples. The method of Waring and Bremner (1964) was modified by adding O_2 free N_2 headspace gas instead of filling the entire serum bottles with H_2O. Triplicate glass serum bottles were prepared by adding ~ 5 g of moist soil and 5 ml of distilled, de-ionized water. Bottles were capped with butyl rubber stoppers and sealed with aluminum crimps. The headspace was evacuated at –85 kPascals and replaced with 99.99% O_2-free N_2 gas. For the respective treatments, NO_3^- as KNO_3 and SO_4^{-2} as K_2SO_4 were added on an electron equivalent basis and at levels, which were determined in preliminary investigations to be in excess assuming a 15 day incubation period and a 10 g wet weight sample. One-half ml of the respective solutions were applied to the individual treatments at start of the incubation and again, at t = 8 d. The concentrations in the sample soil solution of respective electron acceptors at time of spike were 80 mg NO_3-N L^{-1} and 115 mg SO_4-S L^{-1} for the nitrate and sulfate reducing conditions respectively. Serum bottles were incubated in the dark at 30°C for 15 d. A set of triplicate, time zero controls were extracted with 2 *M* KCL at the start of the incubation period.

In preparation for incubation under methanogenic conditions detritus and soil samples (including the controls) were pre-incubated in the dark under anaerobic conditions at 30°C. Headspace gas was periodically monitored by collection of a 50 µl gas sample. Methane concentrations were determined using a Shimadzu 8 AIF gas chromatograph equipped with a flame ionization detector (110°C) with N_2 as the carrier gas and a stainless steel Carboxen 1000 column (Supelco, Inc., Bellefonte, PA),

maintained at 160°C in order to determine the onset of the production of CH_4. The pre-incubation period (length of time the controls were incubated until CH_4 appeared) averaged 4 d for detritus and the 0-10 cm soil depth while averaging 9 d for the 10-30 cm soil interval. The pre-incubation period was necessary to allow the consumption of residual O_2, and NO_3^-, and to allow for the establishment of an active methanogenic microbial consortium.

Randomly selected, serum bottles were monitored over the course of incubation to insure continuous anaerobic conditions. Headspace gas was sampled by withdrawing 100 ul of gas and determining O_2 content by using a Shimadzu 8 AIF gas chromatograph equipped with a thermal conductivity detector with He as the carrier gas and a stainless steel column packed with molecular sieve 5A (Supelco, Inc.), maintained at 30°C. All samples were extracted with 30 ml of 2 *M* KCL at the terminus of the incubation period (15 d). Bottles were shaken for 1 h on a longitudinal shaker and centrifuged for 10 min at 6000 rpm. The supernatant was filtered through Whatman #42 filter paper, collected in 25 ml scintillation vials and refrigerated at 4°C for subsequent automated, colorometric analysis of NH_4^+ (EPA Method 351.2, 1993). Bottle incubations have routinely been used to investigate net N-mineralization rates of detritus and soil under anaerobic conditions (Waring and Bremner, 1964; Satathchandra, et al., 1989, Joegensen et al., 1990; Perucci, 1990; and Gale et al., 1992).

Substrate Induced Nitrogen Mineralization (SINM)

Differences in net N mineralization can be investigated over short time periods (h) with simple substrates (amino acids) because the rate limiting steps of SOM breakdown and decomposition have been removed by providing a readily hydrolyzable substrate. The C:N ratio of various amino acids is much lower than the C:N ratio of the microbial pool resulting in substantial net N release (Alef and Kleiner, 1986). In this fashion, amino acid utilization as a respiratory substrate (electron donor) provides a measure of the activity of the heterotrophic microbial population (Alef et al., 1988, Hopkins et al, 1994; Hopkins et al, 1997).

Substrate induced N mineralization has demonstrated linearity over the short term (a few hours) and has been shown to occur in soils with minimal or no lag phase (Alef and Kleiner, 1986). This method measures the presently active soil microbial population responsible for the final step of organic N mineralization, de-amination, without allowing sufficient time for significant turnover and new biomass production (Alef and Kleiner, 1986; Hopkins and Ferguson, 1994; Franzluebbers et al., 1996).

Soil and detritus samples used in this study were prepared exactly the same as those from the anaerobic mineralization study described earlier. Sample bottles were spiked with NO_3^-, SO_4^{2-} or no additions, and were incubated for 15 d in order to achieve develop microbial communities dominant under NO_3^- reducing, SO_4^{2-} reducing, and methanogenic conditions, respectively. To triplicate samples, 0.5 ml of solution containing 200 mg *L*-alanine ($C_3H_7NO_2$)-N L^{-1} was added by inserting the syringe needle through the rubber stopper, and incubated in the dark at 30°C. Samples were extracted at

the terminus of the 4 h incubation with 30 ml of 2 *M* KCL. Bottles were agitated on a longitudinal shaker for 1 h and vacuum filtered through #42 Whatman filter paper. The supernatant was stored refrigerated at 4°C until subsequent automated colorimetric analysis for NH_4-N (EPA Method 351.2, 1983). The SINM rate was calculated as the difference in extractable NH_4^+ between the spiked incubation and controls samples divided by the 4 h incubation period and dry weight of the sample expressed as mg N kg^{-1} h^{-1}.

Data Analysis

Soil characteristics and microbial processes were statistically related using Pearson's product-moment correlation and regression analysis. All data were checked for homogeneity of variances and log transformed prior to statistical comparisons where appropriate. Analysis of variance (ANOVA) and Fisher's least significant difference (LSD) tests were utilized to make comparisons between treatments using the StatGraphics software program (Manugistics, Inc., Rockville, MD).

Results and Discussion

Soil Characterization

The organic soils contained high weight percent water contents (90-95 %) and low dry weight bulk densities averaging 0.066 (S.E. = 0.004) and 0.088 (S.E. = 0.005) g cm^{-3} for the 0-10 cm and 10-30 cm soil depths, respectively (Table 3-1). Bulk density was not

Table 3-1. Select physiochemical properties of detritus and soils collected from the study transect in WCA-2A. Data are mean values from 4 composited cores collected in March 1997.

Distance	Soil Interval	Bulk Density	Total Carbon	Total Nitrogen	Total Phosphorus	Extractable NH_4^+-N
km		$g\ cm^{-3}$	---------- $mg\ g^{-1}$ ----------		------- $mg\ kg^{-1}$ -------	
1.4	detritus	n.d.	430	27.7	1901	215
2.3	detritus	n.d.	420	27.2	1805	239
3.3	detritus	n.d.	432	25.6	1633	222
4.2	detritus	n.d.	412	23.3	1960	193
5.1	detritus	n.d.	419	21.3	1412	100
7.0	detritus	n.d.	359	24.9	1110	327
8.4	detritus	n.d.	379	26.0	693	232
10.1	detritus	n.d.	425	26.4	445	43.0
1.4	0-10 cm	0.056	378	25.6	1581	67.0
2.3	0-10 cm	0.065	424	29.2	1397	69.3
3.3	0-10 cm	0.062	431	28.1	1324	79.6
4.2	0-10 cm	0.087	424	28.4	877	48.5
5.1	0-10 cm	0.078	418	24.8	974	46.6
7.0	0-10 cm	0.066	393	32	755	52.9
8.4	0-10 cm	0.064	419	27.6	616	121
10.1	0-10 cm	0.048	423	25.4	621	60.0
1.4	10-30 cm	0.097	449	32.5	549	37.7
2.3	10-30 cm	0.105	453	28	368	35.2
3.3	10-30 cm	0.097	473	31	419	36.5
4.2	10-30 cm	0.092	465	30.4	279	40.5
5.1	10-30 cm	0.067	439	31.6	317	27.2
7.0	10-30 cm	0.095	420	25.1	231	17.9
8.4	10-30 cm	0.088	456	27.2	237	22.7
10.1	10-30 cm	0.064	459	32.5	273	40.4

determined for detritus. Total C and N did not vary significantly along the transect, yielding mean values of 410, 414, and 452, g C kg^{-1}, and 25.3, 27.6, and 29.8 g N kg^{-1} respectively, for detritus, 0-10 cm and 10-30 cm soil depths (Table 3-1). Values are similar to those found in previous studies in WCA-2A (Koch and Reddy, 1992; DeBusk et al., 1994). Total C and N were significantly correlated with depth at $P < 0.01$. Total C was significantly correlated with total N ($P < 0.01$; $r = 0.49$), for detritus and soil samples (Table 3-2). The mean C:N ratio was 15.5 (S.E. = 0.31) and demonstrated no significant correlation with distance from the inflow.

Total P for detritus, 0-10, and 10-30 cm soil depths taken separately were significantly negatively correlated (r = -0.95, -0.93 and -0.79, respectively) with distance from inflow (Figure 3-2). Total P was also significantly correlated ($P < 0.01$) with depth ($r = 0.68$) and results of a one-way ANOVA revealed total P was significantly higher ($P < 0.05$) in both detritus and 0-10 cm soil when compared with the underlying 10-30 cm soil. There was also a significant difference in total P content of detritus and the 0-10 cm soil depth.

The results of the SOD measurements on soil samples demonstrated significantly higher O_2 consumption rates in the surface soil. The average SOD rate was 98.2 mg O_2 kg^{-1} h^{-1} (S.E. = 8.8) in the 0-10 cm soil depth and 38.4 mg O_2 kg^{-1} h^{-1} (S.E. = 2.6) for the 10-30 cm depth (data not shown). Soil O_2 demand decreased exponentially with depth (Figure 3-3). The decrease in SOD with depth was attributed to lower microbial activity and less readily available C compounds at lower depths. Oxygen, utilized by

Table 3-2. Product moment correlation matrix for soil characteristic and organic N mineralization rates measured from along the transect in WCA-2A for the March 1997 sampling. For n = 24, r = 0.404 is significant at $P < 0.05$ and r = 0.515 is significant at $P < 0.01$. (MBC=microbial biomass carbon; MBN=microbial biomass nitrogen; Extr NH_4^+=extractable ammonium as N Min-O_2=mineralization under aerobic conditions; Min-NO_3^-=mineralization under nitrate reducing conditions; Min-SO_4^{2-}=mineralization under sulfate reducing conditions; Min-CH_4=mineralization under methanogenic conditions).

	Depth	total C	total N	N:P ratio	total P	MBC	MBN	Extr NH_4^+	MIN-O_2	MIN-NO_3^-	MIN-SO_4^{2-}
total C	0.68										
total N	0.62	0.49									
N:P ratio	0.87	0.60	0.50								
total P	-0.76	-0.42	-0.47	-0.87							
MBC	-0.81	-0.64	-0.61	-0.75	0.73						
MBN	-0.74	-0.63	-0.39	-0.66	0.67	0.88					
Extr NH_4^+	-0.72	-0.57	-0.42	-0.60	0.64	0.89	0.95				
MIN-O_2	-0.81	-0.46	-0.64	-0.69	0.66	0.77	0.58	0.62			
MIN-NO_3^-	-0.66	-0.62	-0.28	-0.51	0.53	0.70	0.80	0.85	0.50		
MIN-SO_4^{2-}	-0.62	-0.70	-0.31	-0.44	0.40	0.72	0.82	0.88	0.46	0.94	
MIN-CH_4	-0.78	-0.53	-0.43	-0.58	0.60	0.83	0.90	0.92	0.73	0.84	0.85

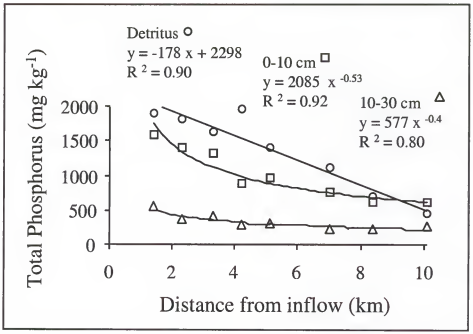

Figure 3-2. Total phosphorus of detritus 0-10 cm and 10-30 cm soil depth from along the study transect in WCA-2A. Samples were collected March 1997.

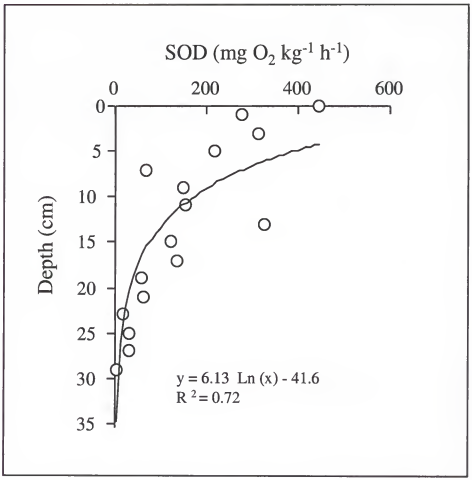

Figure 3-3. Soil oxygen demand (SOD) profile at station 2 in WCA-2A.

heterotrophs as an electron acceptor, is consumed rapidly during microbial respiration in the surface soil. The high oxygen demand of the surface soil prevents O_2 from diffusing downward from the water column into the sediments, assuring the bulk of the wetland soil remains anaerobic. The higher concentration of labile or readily available C compounds in the surface was evidenced by higher microbial respiration (Debusk and Reddy, 1998) and concomitant O_2 depletion. These results support earlier findings of increased recalcitrance or high lignin:cellulose content of organic soils in WCA-2A with increasing depth (DeBusk 1996). In addition, the paucity of O_2 in the soil profile underscores the importance that alternate inorganic electron acceptors can have in mediating the mineralization of organic nitrogen in wetlands.

Extractable NH_4^+ was significantly negatively correlated ($r = -0.72$; $P < 0.01$) with depth, averaging 196, 68, and 32 mg N kg^{-1} for the detrital, 0-10, and 10-30 cm soil depths, respectively. Extractable NH_4^+ was also significantly correlated ($r = 0.64$; $P < 0.01$) with total P (Table 3-2). These results suggest that elevated extractable NH_4^+ concentrations might be useful as a biogeochemical indicator for determining the extent of influence of P loading in wetlands.

Microbial Biomass

Microbial biomass C and N were significantly ($P < 0.01$) correlated with total P ($r = 0.73$; 0.67, respectively). Both microbial biomass C and N were also significantly negatively correlated with depth ($r = -.081$; - 0.74, respectively; Table 3-2). The MBC and MBN were also significantly correlated ($r = 0.88$) with each other at $P < 0.01$. The best-fit linear model of microbial C and N returned an average C:N ratio of 12.9 for the

microbial pool (Figure 3-4). The average C:N ratio increased with depth, suggesting an increasing limitation of inorganic N to the microbial pool with depth. However, differences in the C:N ratio with depth could also be attributed to a change in the overall functional microbial pool composition.

Microbial biomass C and N were both significantly correlated at $P < 0.01$ with extractable NH_4^+ ($r = 0.89$; 0.95, respectively). Extractable NH_4^+ appears to be an excellent indicator of the size of microbial pool measured by chloroform-fumigation extraction (Figure 3-5).

Potential Mineralization Rates

There were significant differences in organic N mineralization rates of native organic matter with depth under each of the aerobic, NO_3^- reducing, SO_4^{2-} reducing, and methanogenic conditions. Under aerobic conditions, organic N mineralization rates averaged 237, 143, and 75 mg N kg^{-1} d^{-1} for detritus, 0-10 cm and 10-30 cm soil depths, respectively (Table 3-3). Rates of N mineralization with depth also followed a predictable pattern for NO_3^- reducing, SO_4^{2-} reducing, and methanogenic conditions with significantly highest rates in the detritus, significantly lower rates in the 0-10 cm soil depth and lowest rates found in the 10-30 cm soil depth (Table 3-3). These results suggest that the surficial detritus may potentially provide the greatest source of inorganic N among the soil compartments sampled. Previous studies have also noted decreased mineralization with increasing soil depths (Franzluebbers et al., 1995, Hossain et al, 1995).

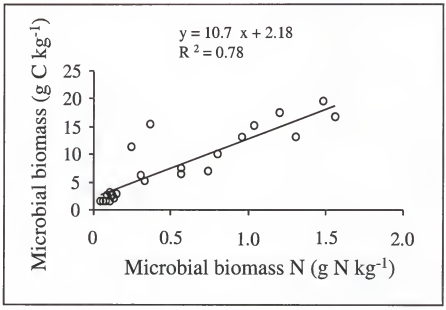

Figure 3-4. Microbial biomass C vs N for soil and detritus from the transect in WCA-2A.

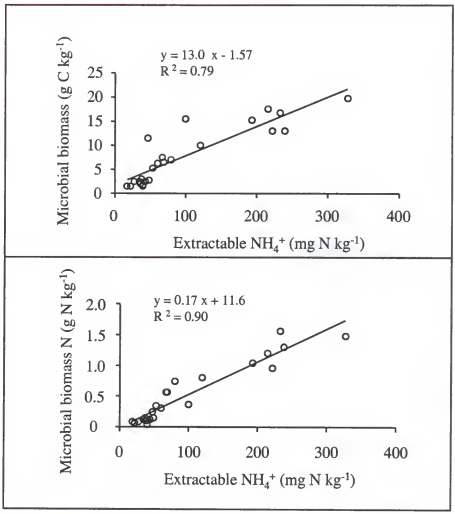

Figure 3-5. Microbial biomass C and N vs extractable NH_4-N for soil and detritus from the transect in WCA-2A.

Table 3-3. Potential N mineralization rates under dominance of various electron acceptors for soils and detritus from WCA-2A. Values are means of 8 station followed by one standard error. Different letters signify a significant difference in mineralization rate with respect to depth for each condition.

Electron Acceptor	Depth Interval	Mineralization Rate	
	cm	mg N kg^{-1} d^{-1}	
O_2	Detritus	237 (26.1)	a
O_2	0-10	143 (8.37)	b
O_2	10-30	74.9 (5.62)	c
NO_3^-	Detritus	59.5 (12.0)	a
NO_3^-	0-10	17.1 (6.29)	b
NO_3^-	10-30	3.95 (1.13)	c
SO_4^{2-}	Detritus	36.0 (7.87)	a
SO_4^{2-}	0-10	8.94 (3.74)	b
SO_4^{2-}	10-30	3.05 (1.46)	c
HCO_3^-	Detritus	19.3 (1.95)	a
HCO_3^-	0-10	4.54 (1.34)	b
HCO_3^-	10-30	1.24 (0.16)	c

There was a significant effect of inorganic electron acceptor dominance on organic N mineralization rates. Each soil depth was examined separately as a significant difference in organic N mineralization rates existed among soil intervals. Organic N mineralization rates in the detrital layer averaged 237, 59.5, 36.0 and 19.3 mg N kg^{-1} d^{-1} under aerobic, NO_3^- reducing, SO_4^{2-} reducing, and methanogenic conditions, respectively, and were significantly different for each conditions (Table 3-4). Rates of N mineralization under the four aforementioned conditions for both the 0-10 and 10-30 cm soil depths followed a somewhat similar pattern. Organic N mineralization rates under aerobic conditions were significantly higher than all other electron acceptors, while there was no significant difference in rates between NO_3^- and SO_4^{2-} reducing conditions. Mineralization rates under NO_3^- reducing conditions were significantly greater than rates under methanogenic conditions, while organic N mineralization rates under SO_4^{2-} reducing and methanogenic conditions were not significantly different from one another (Table 3-4). Similar results have been observed by others for an organic rich wetland soil (McLatchey and Reddy, 1998).

Rates of mineralization under dominance of various electron acceptors were significantly correlated with one another (Table 3-2). Aerobic N mineralization rates were significantly correlated with rates under NO_3^- reducing ($r = 0.59$), SO_4^{2-} reducing ($r = 0.55$) and methanogenic ($r = 0.81$) conditions at $P < 0.01$, respectively. The less strong correlations of aerobic vs both NO_3^- and SO_4^{2-} reducing conditions are due to the non-linearity of the relationship between rates. The equations of the best-fit line for these

Table 3-4. N mineralization rates for detritus and soil collected from WCA-2A. Different letters signify significant differences between rates under dominance of different electron acceptors at each soil depth.

Electron	Mineralization Rate (mg N kg^{-1} d^{-1})		
Acceptor	Detritus	0-10 cm	10-30 cm
O_2	237 a	143 a	74.9 a
NO_3^-	59.5 b	17.1 b	3.95 b
SO_4^{-2}	36.0 c	8.94 b c	3.05 bc
HCO_3^-	19.3 d	4.54 c	1.24 c

cases are exponential of the form y = constant * e $^{(m x)}$ (Figure 3-6). Standard correlation procedures utilize mathematically equivalent calculations for calculating coefficients of determination based on linear relationships. The R^2 values improved to 0.62 and 0.53 for mineralization under NO_3^- and SO_4^{2-} reducing conditions respectively, compared with aerobic conditions when employing a nonlinear equation (Figure 3-6). Comparisons of aerobic N mineralization rates of soil and detritus with rates under methanogenic conditions were best described by a linear equation (Figure 3-6). These results point out the existence of a variety of functional microbial groups viable in the soil (Drake et al., 1996) as well as demonstrating that each functional microbial group can play a significant role in organic N mineralization in wetland soils.

Future work should be concentrated on identifying the size of the specific functional microbial component of the total pool present and active in the soil. A shift in the specific microbial pool composition from an impacted area compared with an unimpacted soil might give an indication of ecosystem change and function at the microbial level. In addition, this technique might be useful in helping to determine the percent functional recovery of a formerly impacted wetland to pre-impact status.

Rates of organic N mineralization under aerobic conditions were significantly correlated to total P (r = 0.57), MBC (r = 0.77), MBN (r = 0.58), and extractable NH_4^+ (r = 0.86) at $P < 0.01$ (Table 3.2). Extractable NH_4^+ concentrations were found to explain 74 % of the variability in aerobic mineralization rates and is therefore, a useful indicator, as well as an easily measured parameter (Figure 3-7).

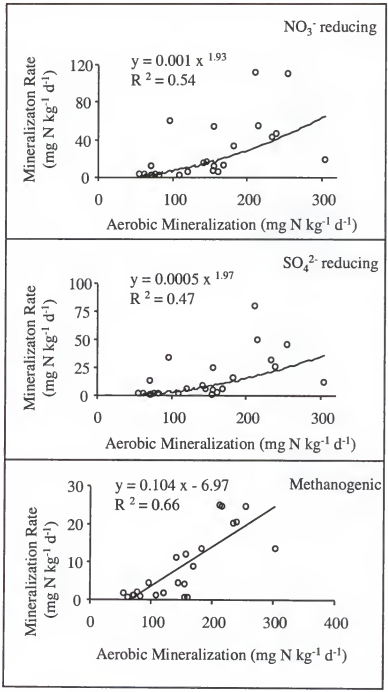

Figure 3-6. Rates of potential N mineralization under nitrate reducing, sulfate reducing, and methanogenic conditions plotted as a funtion of N mineralization under aerobic conditons for detritus and soils.

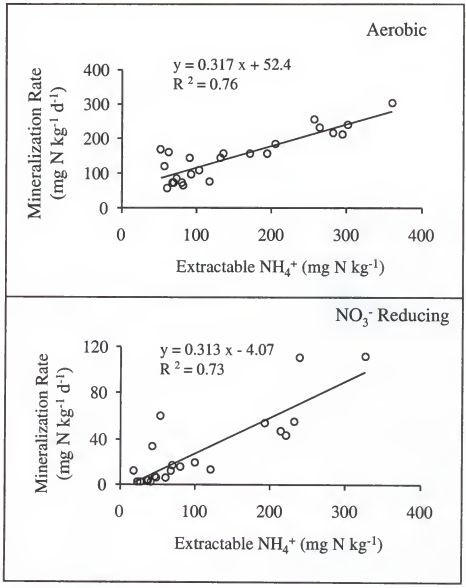

Figure 3-7. Rates of potential N mineralization under aerobic and nitrate reducing conditions vs extractable NH_4-N for detritus and soils collected from WCA-2A.

Rates under NO_3^- reducing conditions were significantly correlated to total P (r = 0.53), MBC (r = 0.70), MBN (r = 0.80), and extractable NH_4^+ (r = 0.85) at $P < 0.01$ (Table 3.2). Extractable NH_4^+ was again, the strongest indicator of organic N mineralization rates with the equation of the best fit line explaining 73 % of the variability (Figure 3-7). The coefficient of determination did not improve significantly by including a microbial term or total P term in a multiple regression equation and therefore those terms were omitted.

A similar relationship was seen under SO_4^{2-} reducing conditions which were significantly correlated to total P (r = 0.40) at $P < 0.05$, and MBC (r = 0.72), MBN (r = 0.82), and extractable NH_4^+ (r = 0.88) at $P < 0.01$ (Table 3-2). Extractable NH_4^+ was the strongest indicator of N mineralization rates with the model equation explaining 77 % of the variability (Figure 3-8). As with the other comparisons, the coefficient of determination did not improve significantly by including a microbial or total P term in a multiple regression equation.

The N mineralization rates under methanogenic conditions were the most strongly correlated rates with measured soil characteristics, including total P (r = 0.60), MBC (r = 0.83), MBN (r = 0.90), and extractable NH_4^+ (r = 0.92) at $P < 0.01$ (Table 3-2). Again, extractable NH_4^+ was the strongest indicator mineralization rates and the equation of the best fit line explained 85 % of the variability in N mineralization under methanogenic conditions (Figure 3-8).

These results suggest that, for the waterlogged, highly reduced organic soils of WCA-2A, extractable NH_4^+ was an excellent indicator of potential organic N

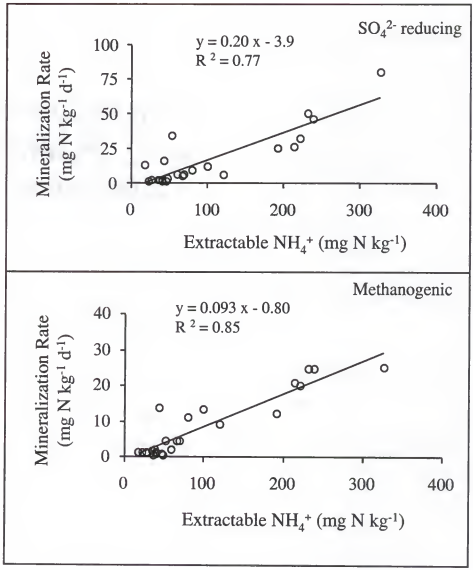

Figure 3-8. Rates of potential N mineralization under sulfate reducing and methanogenic conditions vs extractable NH_4-N for detritus and soils collected from WCA-2A.

mineralization of soil and detritus under aerobic, NO_3^- reducing, SO_4^{2-} reducing, and methanogenic conditions. In addition, rates were strongly correlated with the size of the microbial pool suggesting microbial pool size was a relatively sensitive indicator of the heterotrophic microbial pool activity and, consequently, nutrient cycling.

Substrate induced nitrogen mineralization (SINM)

The SINM was measured under anaerobic conditions to determine the relative activity of the portion of the microbial pool responsible for the final steps in organic N mineralization (de-amination) under NO_3^- reducing, SO_4^{2-} reducing, and methanogenic conditions. The rates of SINM under NO_3^- reducing and methanogenic conditions were significantly correlated with distance from the inflow (Table 3-5). Both SINM rates under NO_3^- and SO_4^{2-} reducing conditions were significantly ($P < 0.05$) negatively correlated with depth and SINM under methanogenic conditions was weakly negatively correlated with depth at $P < 0.10$. Similar results of higher N mineralization rates in surface than subsurface soils have been seen for native organic matter by others (Franzluebbers et al., 1996; Hossain et al., 1995).

There were no significant differences in SINM among anaerobic electron acceptor conditions (Table 3-6). In order to remove N in the structure of *L*-Alanine, a simple deamination is required. It is likely that the effect of electron acceptors on organic N mineralization is linked only to the return of energy in breaking the C-N bonds in the larger organic molecules, as was the case in mineralization of the larger, more complex

Table 3-5. Product moment correlation matrix for soil characteristic and SINM rates measured from along the transect in WCA-2A for the March 1997 sampling. For n = 24, r = 0.404 is significant at $P < 0.05$ and r = 0.515 is significant at at $P < 0.01$. (MBC=microbial biomass carbon; MBN=microbial biomass nitrogen; Extr NH_4^+=extractable ammonium as N; SINM-NO_3^-=mineralization under nitrate reducing conditions; SINM-SO_4^{2-}= mineralization under sulfate reducing conditions; SINM-CH_4=mineralization under methanogenic conditions).

	Distance	Depth	total P	MBC	MBN	SINM-NO_3^-	SINM-SO_4^{2-}
total P	-0.53	-0.76					
MBC	-0.13	-0.81	0.73				
MBN	-0.14	-0.74	0.67	0.88			
SINM-NO_3^-	0.41	-0.42	0.08	0.60	0.62		
SINM-SO_4^{2-}	0.29	-0.44	0.23	0.35	0.38	0.54	
SINM-CH_4	-0.42	-0.37	0.57	0.44	0.19	0.02	0.10

Table 3-6. Potential mineralization rates of alanine. Rates are mean values from 8 stations with one standard error in parentheses.

Sample Interval	N- Mineralization Rate: Dominant Electron Acceptor NO_3^-	SO_4^{-2}	CO_2
	(mg N kg^{-1} h^{-1})		
Detritus	105 (20.5)	118 (24.7)	122 (56.9)
0-10 cm	57.4 (11.2)	57.1 (12.8)	72.3 (16.3)
10-30 cm	51.5 (12.7)	49.6 (13.4)	34.9 (6.9)

native organic matter molecules. De-amination is not the rate-limiting step in the breakdown of organic N and therefore, rates were not affected by dominance of any particular electron acceptor. Only SINM rates under NO_3^- reducing conditions were significantly correlated with MBN. McLatchey and Reddy (1998) reported that the size and activity of the microbial biomass decreased under influence of the inorganic electron acceptors; NO_3^-, SO_4^{2-} and H_2CO_3, respectively. It is likely the microbial biomass changed in both size and composition during the 15 d incubation to establish each reducing condition, and consequently, was responsible for the weak or non significant correlations of SINM with the initial soil MBN under both SO_4^{2-} reducing and methanogenic conditions (Table 3-5). Additionally, the presence and concentration of active extracellular enzymes might be more significant in regulating N release from amino acids in soils. Therefore, SINM did not provide any useful indication of the relative rates of organic N mineralization under aerobic, NO_3^- reducing, SO_4^{2-} reducing and methanogenic conditions.

Future work should concentrate on determining the effect the location of the N in different amino acids has on N mineralization rates. In this fashion, mineralization rates of amino acids which require breakage of the C-N-C linkages might be affected by different electron acceptor availability and could be used to further define the role of functional microbial consortia on the release of inorganic N in wetland soils.

Ecological Implications

The importance of various electron acceptors on the N mineralization rate of native soil was demonstrated in laboratory incubations. The release of inorganic N was highest under aerobic conditions with lowest rates were seen under methanogenic conditions. Organic N mineralization rates under NO_3^- and SO_4^{2-} conditions were intermediate. Several issues should be highlighted in order to correctly apply the laboratory data to the field, however. First, it appears from the data that, any condition which exposes the surface of the soil to direct contact with O_2 in the atmosphere will cause considerable soil oxidation and release of inorganic N. There were considerable periods during 1995, 1996, and 1997 when the soil surface was exposed at station 2 (Figure 3-9). During these "dry periods", the majority of inorganic N release can be expected to take place, due to the disparity of aerobic N mineralization rates when compared to those under any of the three anaerobic conditions (Figure 3-8). However, only the top-most soil layers are likely to undergo aerobic decomposition due to the high C content and SOD of these organic soils.

In determining the relative contribution of the anaerobic conditions on N liberation from the soil organic matter, one must consider the results from the chapter 5 study on denitrification. The influence of high NO_3^- loading from agricultural drainage waters is limited to ~ 2 km from the inflow points, and then only to the detrital and 0-10 cm soil depths. Consequently, organic N mineralization under NO_3^- reducing conditions is restricted to areas along the transect proximal to the surface water inflow points. The concentrations of SO_4^{2-} are also highest at the inflow points evidenced by both the strong

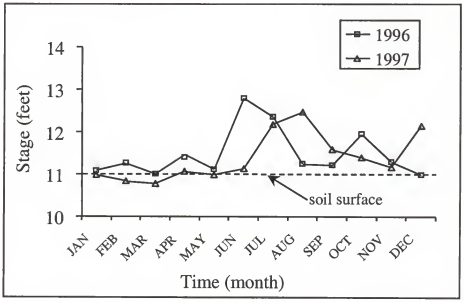

Figure 3-9. Stage data recorded at station 2 in WCA-2A. At stage height ~ 11 feet at the gauge location, > 85 % of the surrounding soil surface is exposed to the atmosphere.

odor of H_2S gas in the cattail areas and supporting water quality data from the SFWMD. Therefore, the cattail areas are likely to undergo relatively high organic N mineralization rates *in situ* due to the availability of O_2 part of the year and a nearly continuos supply of NO_2^- and SO_4^{2-} during the summer months when hydraulic loading is highest.

The natural sawgrass marsh located in the interior of WCA-2A contains soil with very low denitrifying enzyme activity, which suggests very little NO_3^- present. The SO_4^{2-} concentration is also significantly lower in the porewater than found in the cattail regions. The presence of open water dominated by periphyton, however, provides a mechanism of transporting O_2 into the soil to stimulate the inorganic N release within these areas. During the daylight hours, photosynthetic activity is high and redox profiles (data not shown) have demonstrated aerobic conditions down into the detrital layer and even reaching the surface of the peat soil. This cyclic mechanism occurs daily, with low bottom water O_2 levels experienced during the night. The oxidation of the detrital material might provide an important inorganic N source for the macrophytes and is most likely, partly responsible for the low organic soil accretion rates in these areas.

Conclusions

Several soil characteristics were significantly correlated with organic N mineralization rates of native soil organic matter including MBC, MBN, and extractable NH_4^+. Extractable NH_4^+ of soils and detritus was by far, the most sensitive and reliable indicator for mineralization rates under aerobic, NO_3^- reducing, SO_4^{2-} reducing, and methanogenic conditions. The concentration of extractable NH_4^+ was found to be a sensitive indicator for potential organic N mineralization (PMN). This relationship holds

true in Northern Everglades soils due to the high SOD of these organic soils, which prevents the diffusion of O_2 from the floodwater into the soil, thereby halting the autolithotrophic conversion of NH_4^+ to NO_3^- deep in the soil profile. Therefore, only through diffusion upward under a concentration gradient and subsequent denitrification in the surface soils is N lost to the atmosphere as N_2.

The dominance of inorganic electron acceptors was found to have a controlling factor in organic N mineralization rates of native soil organic matter. Rates of N mineralization of soil ranged from highest rates under aerobic conditions and significantly lower rates under each of NO_3^- reducing and SO_4^{2-} reducing conditions and lowest under methanogenic conditions for soil and detritus. There appeared to be no electron acceptor effect on SINM rates in soil and detritus.

These data on potential rates of organic N mineralization can be utilized to determine the potential effect of altered hydrology and increased loading of dissolved NO_3^- and SO_4^{2-} on the biogeochemical cycling of N in these soils. A decrease in surface water hydraulic loading to WCA-2A could increase organic N mineralization rates. Increased mass loading rates of NO_3^- and SO_4^{2-} in surface or groundwater could also potentially increase ammonification rates, thereby altering the overall balance of N in this 44,000 hectare wetland.

CHAPTER 4
INFLUENCE OF PHOSPHORUS LOADING ON NITRIFICATION-DENITRIFICATION PROCESSES

Introduction

Wetlands are widely utilized for their ability to remove N from floodwater. This function is due to the existence of two coupled processes; nitrification and denitrification. Nitrification, an obligate aerobic process, is the autolithotrophic conversion of NH_4^+ to NO_3^-. Nitrification is the biological oxidation of NH_4^+ to NO_2^- and NO_3^-. The chemoautotrophic bacteria couple the oxidation of NH_4^+ to electron transport phosphorylation and utilize CO_2 to synthesize required cellular components. The oxidation state of N is increased from –3 for NH_4^+ to +3 (NO_2^-) or +5 (NO_3^-).

Nitrification is regulated at the cellular level by NH_4^+ concentration and aeration status and is usually the rate limiting process in the conversion of NH_4^+ to N_2 in continuously flooded wetlands due to the obligate aerobic requirement (Robertson, 1989). Very little O_2 actually diffuses into a flooded, organic rich soil profile due to the slower diffusion rate of O_2 in water (10,000 times slower than in air) and high soil oxygen demand (SOD). These two factors allow NH_4^+ concentrations to build up in wetland soils. The concentration gradient, and soil characteristics, such as porosity and cation exchange capacity control transport out of the soil. The slow diffusion of NH_4^+ out of the wetland soil allows the inorganic N to be utilized by macrophytes and soil microbial

communities (Reddy and Patrick, 1980). Ammonium, once diffused into the overlying, oxygenated water column becomes nitrified, increasing the NO_3^+ concentration of the floodwater (Reddy and Patrick, 1984). Diffusion control over the transport of N between the bulk aerobic and anaerobic zones in wetlands can mediate the rate of loss of N from the system.

Biological denitrification is the microbial-mediated reduction of nitrogenous oxides to N_2O and N_2 gas. The facultative microbial populations reduce nitrogenous oxides in a stepwise fashion as membrane bound enzyme systems are engaged in electron transport phosphorylation (Tiedje, 1982). The oxidation state of N, in reduction of NO_3^- to N_2 gas, decreases from +5 to 0, respectively.

Denitrification rates are controlled by three factors at the cellular level, available carbon, NO_3^- concentration and the soil aeration status (Robertson, 1989). There is a well established correlation for a soil's capacity for denitrification and the organic carbon content of the soil (Burton and Beauchamp, 1985). Carbon is utilized as the electron donor and must be present in a readily utilizable form by the microbial population. The presence or absence of O_2 in the soil profile limits the extent to which denitrification can occur. Oxygen has an inhibitory effect on the faculative anaerobes as these organisms prefer O_2 as the electron acceptor of choice. Only when the O_2 content is low, do denitrifiers produce significant quantities of nitrate reducing enzymes. Nitrate is a principal regulator of denitrification. The denitrifiers must have NO_3^- available, in order for respiration to occur in low O_2 environments. In general, wetland soils have higher organic C content and a low O_2 status due to poor drainage and presence of floodwater, which leaves NO_3^- as the lone proximal regulator of denitrification in these environments.

Nitrate is supplied through a series of microbial-mediated reactions from the organic N pool through N mineralization and subsequent nitrification. The major forms

of N in flooded wetland soils are organic N and NH_4^+. Therefore, the rate of ammonification and nitrification can limit the availability of NO_3^- for denitrification (Reddy and Graetz, 1988). As NO_3^- diffuses downward into the underlying, anaerobic soil, it is denitrified to N_2O or N_2 gas (Reddy and Patrick, 1984). Nitrate can also be supplied to wetland systems from exogenous sources. Consequently, NO_3^- reduction rates are highest proximal to source inputs into the wetland or riparian zones, decreasing significantly with increasing distance from the loading point (Cooper, 1990; Gale et al., 1993; Schipper et al., 1993).

Study Area

The Florida Everglades are currently affected by nutrient loading from urban and agricultural surface runoff. Most notably, this impact is seen in the Water Conservation Areas, one of the major hydrologic units of the Everglades (DeBusk et al, 1994). Water Conservation Area 2A has been receiving nutrient-laden (N and P) drainage waters for the past 40 years. Peat and nutrient (organic C, N and P) accretion rates have increased in areas receiving surface drainage water (Koch and Reddy, 1992; Craft and Richardson 1993). Most notably, the impact of anthropogenic nutrient loading is documented in the spatial distribution of surface soil total P. Total P concentration grades from a high of ~1600 mg kg^{-1} at the surface water inflow points to a background concentration of ~400 mg kg^{-1} in unimpacted areas, located in the interior of the marsh (Koch and Reddy, 1992; Reddy et al., 1993; DeBusk et al., 1994). Gradients in N and P of water column and periphyton tissue have also been documented along the same eutrophic transect in WCA-2A (McCormick and O'Dell, 1996).

Historically an oligotrophic, P-limited sawgrass (*Cladium jamaicense* Crantz) marsh, the vegetation began a shift towards a dominant cattail (*Typha domingensis* Pers.) vegetative community proximal to all surface water inflow points (Davis, 1991; Craft and Richardson, 1997). The timing of vegetative replacement coincided with the initiation of management of surface water pumped primarily from the canal network draining the Everglades Agricultural Area (EAA) approximately 40 years before present.

Objectives

The objectives of this study were to (1) determine the spatial variability (depth and distance) of initial and potential nitrification rates of soil, (2) measure potential rates of denitrification and (3) determine the relationship between measured soil characteristics (including total P) and potential nitrification-denitrification rates.

Materials and Methods

Experimental Design

Eight stations were located along a 10 km transect originating from the S-10C inflow water control structure. The study transect spanned the marsh from a primary water control inflow structure (S-10C), southward across the dominant cattail (*Typha sp.)* vegetation and terminated approximately 10 km into the natural (unimpacted) marsh characterized by stunted stands of sawgrass (*Cladium* sp.) separated by shallow sloughs dominated by floating and attached cyanobacterial mats (Figure 4-1). Sampling stations

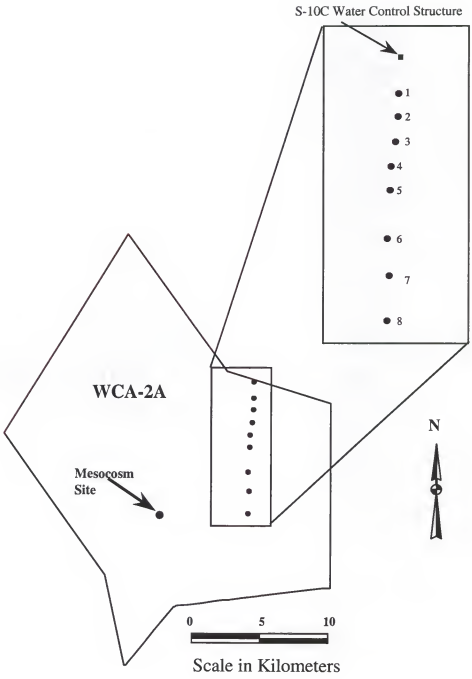

Figure 4-1. Station locations along soil phosphorous gradient in WCA-2A (south of S-10C water control structure) used in the study.

were located at distances of 1.4, 2.3, 3.3, 4.2, 5.1, 7.0, 8.4 and 10.1 km. Water depths varied seasonally from <2 cm to ~ 2 m along the transect length. Sampling along the transect was not designed to identify differences between individual stations, but rather to investigate the gradient or trends between soil characteristics and selected N microbial processes, including initial and potential nitrification and potential denitrification rates.

Soil Sampling

Two soil cores were collected within 1 m of one another at each station by driving a 10 cm diameter aluminum irrigation pipe into the soil. A probe was inserted into each core to verify that negligible (<5%) compaction had occurred during coring. Cores were sealed, removed from the ground, immediately extruded and separated into soil intervals (0-10 and 10-30 cm) in the field. Detrital plant litter was also collected at each station. Detritus consisted of recognizable, loosely associated cattail or sawgrass plant material lying on the surface of the more compact, brown, peat soil. The detritus layer varied in thickness from <1 cm in the sawgrass areas to >25 cm at the cattail stations closest to the inflow. Each sample interval was well mixed to yield a representative and homogenous sample from each station.

Field sampling of detritus and soil was conducted in October 1997. Cores were sectioned in the field, placed in plastic bags, and transported to the laboratory on ice. Samples were transferred into 2 L polyethylene containers with 24 h of collection and stored refrigerated at 4°C until subsequent characterization.

Soil Characterization

Percent moisture (dry weight basis) was determined by drying ~ 20 g of a field moist sub-sample in a forced -air drying oven at 70°C. Bulk density was calculated for the soil intervals on a dry weight basis. Bulk density was not determined for detritus. Total C and N content of detritus and soils was determined on dried, ground samples using a Carlo-Erba NA-1500 CNS Analyzer (Haak-Buchler Instruments, Saddlebrook, NJ). Total P analysis was performed on separate sub-samples prepared by nitric-perchloric acid digestion (Kuo, S. 1996). Total P in the digestate was determined using an automated ascorbic acid method (Method 365.4, USEPA, 1983).

Soil oxygen demand (SOD) was determined for the 0-10 and 10-30 cm soil depths from the February 1996 sampling by adding 10 gm wet soil to 300 ml of distilled, de-ionized water in a capped, continuously stirred BOD bottle. Dissolved O_2 was monitored using a YSI Model 58 oxygen meter with probe (Yellow Springs, CO). SOD was calculated as the difference in O_2 saturated water at time zero minus measured dissolved O_2 after 8 h divided by the dry weight of the soil sample (APHA, 1992; 2-64,65)

Extractable NH_4^+ was determined by shaking triplicate soil samples with 25 ml of 2 *M* KCL at a ratio of approximately 1:40 (g dry soil:extractant) for 1 h on a longitudinal shaker. Samples were centrifuged for 10 min and vacuum-filtered through Whatman #42 filter paper. The supernatant was collected and refrigerated at 4°C and determined colorimetrically for NH_4-N (EPA method 351.2, 1983).

The initial microbial biomass C (MBC) was determined by the 24 h fumigation-extraction technique after Vance et al. (1987). Three replicate samples were used for each soil interval and station. Samples were extracted with 20 ml of 0.5 *M* K_2SO_4,

agitated for 30 min on a longitudinal shaker and vacuum filtered through #42 Whatman filter paper. The supernatant was collected and refrigerated at 4°C until analyzed on a Dohrman TOC analyzer. Microbial biomass carbon was determined by subtracting the extractable total organic carbon (TOC) in the triplicate controls from the triplicate chloroform-treated samples. An extraction efficiency (k_{EC}) factor of 0.37 was applied, utilizing the previous calibration for organic soils by Sparling et al. (1990).

Microbial biomass N (MBN) was determined by the fumigation-extraction technique after Brookes et al. (1985). Ten ml of extract from the microbial carbon procedure was subjected to Kjeldahl-N digestion using the salicylic acid modification of Bremner and Mulvaney (1982). Extracts were analyzed for NH_4-N colorometrically (EPA Method 351.2, 1993). Microbial biomass N was determined by subtracting the extractable NH_4-N of the triplicate non-fumigated samples from triplicate fumigated samples. A combined extraction efficiency and k_{EN} value of 0.54 was applied (Brookes et al., 1985).

Initial and Potential Nitrification Rates

Initial and potential nitrification rates were determined for soil and detritus utilizing constantly stirred reactors. The reactor samples were prepared by placing ~ 300 g wet weight of soils and detritus in triplicate 1 L Erlenmeyer glass flasks and mixing with 400 ml of water. Flasks were placed on magnetic stirrers and equipped with floating, magnetic stir bars. Continual mixing, in concert with continuous aeration with room air, using an aquarium pump connected to glass tubing inserted through a butyl rubber stopper in each flask, helped maintain aerobic conditions in the reactors. The

redox status of the soil slurries was monitored using a Fisherbrand Accumet 1002 combination electrode with platinum band (Fisher Scientific, Pittsburgh, PA) and temperature was recorded with mercury thermometers. The average temperature of the reactors was ~ 30°C. Flasks were wrapped with opaque paper to shield the samples from direct light.

Samples were collected from each reactor daily and extracted with 10 ml of 2 M KCL. Extracts were mixed on a longitudinal shaker for 30 min, centrifuged for 10 min at 6000 rpm, and filtered through # 42 Whatman filter paper. The supernatant was refrigerated at 4°C for subsequent, automated, colorometric analysis of NH_4^+ (EPA Method 350.1, 1983) and NO_3^- (EPA Method 353.2, 1983).

Initial nitrification rates (k_1) were calculated using linear regression over time for the first two days of NO_3^- appearance. The potential nitrification rates (k_m) were calculated by taking the slope of the steepest part of the NO_3^- vs time curve over the course of the experiment. The time lag or delay (t_d) until maximun nitrification was determined from extending a straight line from the point of maximum nitrification to the x-axis (Hue and Adams, 1984).

Denitrifying Potential

Laboratory incubations were performed in order to determine the denitrifying potential of detritus and soils collected along the transect in October 1997. Approximately 20 ml of soil slurry was obtained from each reactor after 25 d, placed in glass serum bottle, and sealed with butyl rubber septa and aluminum crimp caps. Headspace air was evacuated to -85 kPascals and replaced with 99.99 % O_2-free N_2 gas

to achieve anaerobic conditions. Approximately 15 % of the headspace N_2 was replaced with acetylene gas (C_2H_2) (Balderston et al., 1976; and Yoshinari & Knowles, 1976). Bottles were shaken on a longitudinal shaker in the dark at 30°C for 36 h. Headspace gas was sampled at ~ 2, 8, 12, 24, and 36 h. Nitrous oxide production was adjusted for N_2O dissolved in the aqueous phase using Bunsen absorption coefficients (Tiedje, 1982). The potential denitrification rate was calculated from the steepest portion of curve produced when cumulative N_2O evolution was plotted against time.

Data Analysis

Soil characteristics and microbial processes were statistically related using Pearson's product-moment correlation and regression. All data were checked for homogeneity of variances and log transformed prior to statistical comparisons where appropriate. Analysis of variance (ANOVA), student's T, and Fisher's Least Significant Difference (LSD) tests were utilized to make comparisons between treatment (Manugistics, Inc., Rockville, MD).

Results and Discussion

Soil Characterization

The organic soils contained high water contents (90-95 %) and low dry weight bulk densities averaging 0.066 and 0.088 g cm^{-3} for the 0-10 cm and 10-30 cm soil intervals, respectively. Total C and N did not vary significantly along the transect,

yielding mean values of 426, 439, and 444, g C kg^{-1}, and 21.9, 26.5, and 27.5 g N kg^{-1} respectively, for detritus, 0-10 cm and 10-30 cm soil intervals (Table 4-1). Values are similar to those found in previous studies in WCA-2A (Koch and Reddy, 1992; DeBusk et al., 1994). Total C and N of soil were significantly correlated with depth at $P < 0.01$. Total C was significantly correlated with total N ($P < 0.01$; r = 0.45), for all samplings of detritus and soil (Table 4-2). The mean C:N ratio was 17.6 (S.E. = 0.5) and demonstrated no significant correlation with distance from the inflow.

Total P was significantly negatively correlated ($P < 0.01$; r = -0.53) with distance from inflow for all samples (Figure 4-2). Total P for detritus and the 0-10 cm soil depth, taken separately, were significantly negatively correlated with distance (r = -0.78 and -0.93, respectively). Total P concentration in the 10-30 cm soil exhibited no significant trend with distance from inflow, suggesting this soil was the pre-impacted marsh surface. Total P was significantly negatively correlated ($P < 0.01$) with depth (r = - 0.68) and results of a one-way ANOVA revealed total P was significantly higher ($P < 0.05$) in both detritus and 0-10 cm soil when compared with the underlying 10-30 cm soil.

The results of previous SOD measurements on the 0-10 cm and 10-30 cm soil depths demonstrated significantly higher O_2 consumption rates in the surface soil interval with lower rates in the subsurface. The average SOD rate was 98.2 mg O_2 kg^{-1} h^{-1} (S.E. = 8.8) in the 0-10 cm soil interval and 38.4 mg O_2 kg^{-1} h^{-1} (S.E. = 2.6) for the 10-30 cm interval. The decrease in SOD with depth was attributed to 2 factors; lower activity of the microbial pool and less readily available C compounds. Oxygen, utilized by heterotrophs as an electron acceptor, is consumed rapidly during microbial respiration in the surface soil. The concentration of readily available C compounds was evidenced by high microbial respiration rates (DeBusk and Reddy, 1998) and concomitant O_2 depletion

Table 4-1. Select physiochemical properties of detritus and soils collected from along the study transect in WCA-2A. Data are mean values from 4 composited cores collected in October 1997.

Station Number	Sample Interval	Distance	Total Carbon	Total Nitrogen	Total Phosphorus	Extractable NH_4-N
		km	------- mg g^{-1} -------		------- mg kg^{-1} -------	
1	Detritus	1.4	443	26.0	1688	602
2	Detritus	2.3	423	25.0	1328	513
3	Detritus	3.3	437	21.0	1294	530
4	Detritus	4.2	420	20.0	1652	389
5	Detritus	5.1	434	19.0	1209	723
6	Detritus	7.0	440	19.0	1042	589
7	Detritus	8.4	400	24.0	671	563
8	Detritus	10.1	409	21.0	543	411
1	0-10 cm	1.4	437	26.0	1795	270
2	0-10 cm	2.3	454	30.0	1254	266
3	0-10 cm	3.3	423	24.0	1148	183
4	0-10 cm	4.2	445	27.0	1077	125
5	0-10 cm	5.1	442	26.0	1351	343
6	0-10 cm	7.0	442	23.0	802	187
7	0-10 cm	8.4	439	29.0	475	104
8	0-10 cm	10.1	426	27.0	398	113
1	10-30 cm	1.4	443	30.0	514	236
2	10-30 cm	2.3	436	26.0	156	120
3	10-30 cm	3.3	437	28.0	913	166
4	10-30 cm	4.2	448	33.0	288	145
5	10-30 cm	5.1	438	25.0	506	206
6	10-30 cm	7.0	444	27.0	233	137
7	10-30 cm	8.4	453	25.0	195	138
8	10-30 cm	10.1	451	26.0	273	159

Table 4-2. Correlation matrix of soil characteristics and microbial processes for detritus and soil samples collected along the transect in WCA-2A in October 1997 (extract-NH_4^+ = extractable NH_4-N, MBC = microbial biomass carbon; MBN = microbial biomass nitrogen; Initial NIT = initial nitrification rate; Pot NIT = potential nitrification rates; Pot DEN =potential denitrification rate).

	Depth	total C	total N	total P	N:P ratio	extract-NH_4^+	MBC	MBN	Initial NIT	Pot NIT
total C	0.53									
total N	0.60	0.45								
total P	-0.68	-0.13	-0.34							
N:P ratio	0.75	0.30	0.41	-0.83						
extract-NH_4^+	-0.74	-0.39	-0.64	0.56	-0.58					
MBC	-0.63	-0.21	-0.38	0.60	-0.46	0.72				
MBN	-0.65	-0.22	-0.54	0.56	-0.46	0.72	0.92			
Initial NIT	-0.66	-0.48	-0.29	0.39	-0.44	0.70	0.51	0.43		
Pot NIT	-0.22	0.20	0.13	0.51	-0.49	0.10	0.11	0.04	0.22	
Pot DEN	-0.81	-0.18	-0.38	0.85	-0.77	0.50	0.52	0.54	0.39	0.50

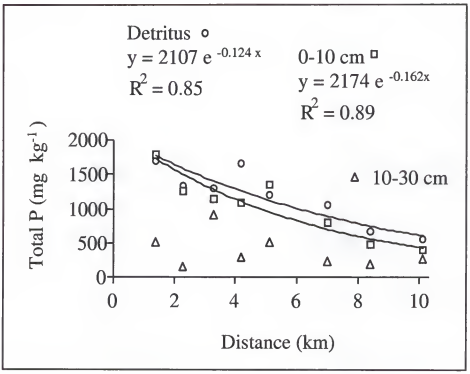

Figure 4-2. Total P vs distance from inflow for detritus, 0-10 cm, and 10-30 cm depth collected from along the transect in WCA-2A for the October 1997 sampling date.

(SOD) in the surface soils. High oxygen demand of the surface soil prevented diffusion of O_2 downward from the water column into the underlying soil, assuring the bulk of the wetland soil remain anaerobic. The juxtaposition of the aerobic water column and anaerobic soil profile underscores the importance of determining coupled nitrification-denitrification processes that can mediate the removal rate of N from the wetland system (Reddy and Patrick, 1984).

Extractable NH_4^+ has been shown to be an excellent indicator of heterotrophic N mineralization potential of these wetland soils due to the anaerobic status of the soil system (Williams and Sparling, 1988; Ross et al., 1995). Consequently, the distribution of extractable NH_4^+ might provide a measure of other microbial processes in wetland soils. Extractable NH_4^+ was significantly negatively correlated ($r = -0.74$; $P < 0.01$) with depth and weakly correlated ($r = 0.56$; $P < 0.01$) with total P (Table 4-2). Decreasing concentrations of extractable NH_4^+ with increasing soil depth have been seen by others (Humphrey and Pluth, 1996).

Microbial Biomass

The size and activity of the microbial pool are important measures of N transformation processes in soils (Wardle, 1992) Microbial biomass C and N were significantly ($P < 0.01$) correlated with total P ($r = 0.60$; 0.56, respectively). Both microbial compartments were also significantly negatively correlated with depth ($r =$ -0.63; - 0.65, respectively; Table 4-2). The MBC averaged 12.1, 2.02, and 0.92 g C kg^{-1} and MBN averaged 1093, 299, and 144 mg N kg^{-1} for the detritus, 0-10 cm and 10-30 cm soil depths, respectively.

The MBC and MBN were also significantly correlated (r = 0.92) with one other at $P < 0.01$, demonstrating that either procedure can be effectively utilized in flooded soils in determining relative microbial biomass pool sizes. The best-fit linear model of the microbial C and N returned an average C:N ratio of 9.44 for the microbial pool and is in strong agreement with data obtained previously for soils from WCA-2A (DeBusk, 1996). Extractable NH_4^+ was significantly correlated ($P < 0.01$; r = 0.72) with both MBC and MBN suggesting that extractable NH_4^+ might be utilized as an indicator of heterotrophic potentials in wetland soils (Figure 4-3).

Initial and Potential Nitrification Rates

The distribution of nitrifying organisms in a flooded, wetland soil should be concentrated closest to the surface of the soil, where O_2 in the water column diffuses down into the soil, a requirement for nitrification to proceed. The initial nitrification rates of soil and detritus in WCA-2A were significantly negatively correlated with depth at $P < 0.01$ (r = -0.66). Mean initial nitrification rate of detritus, 0-10 cm, and 10-30 cm soil depths were 28.5 (S.E. = 32.3), 12.8 (S.E. = 4.13), and 2.13 (S.E. = 0.21) mg N kg d^{-1}, respectively (Table 4-3). The differences in initial nitrification rates are likely due to the presence of active nitrifying populations in each soil depth. The presence of active nitrifiers in the subsurface soil (10-30 cm) is likely due to the presence of a small population associated with the rhizosphere of macrophytes, which transport O_2 down into the soil and release it through the root, creating a niche for these specialized organisms

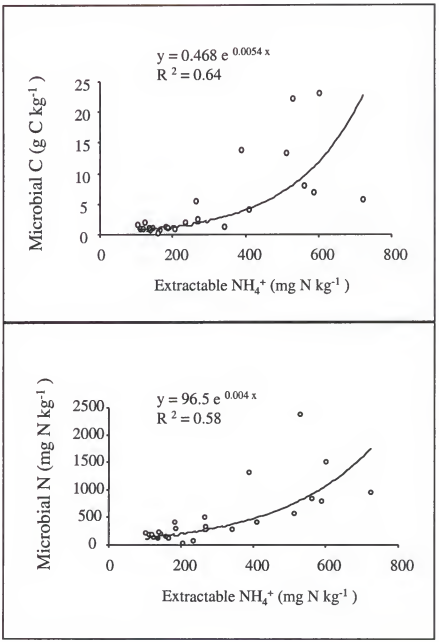

Figure 4-3. Microbial biomass C and N vs extractable NH_4^+ for detritus, 0-10 cm, and 10-30 cm soil depths from along the transect in WCA-2A.

Table 4-3. Initial and potential nitrification rates for soil and detritus collected from along the transect in WCA-2A. Data are mean values (n = 3) and one standard error in parentheses. Time delay is the length of time required to achieve maximum nitrification.

Station Number	Sample Interval	Distance	Initial nitrification rate	Potential nitrification rate	time delay
		km	---------- mg N kg^{-1} d^{-1} ----------		d
1	Detritus	1.4	29.9 (4.2)	322 (88)	3.7
2	Detritus	2.3	51.1 (10)	206 (73)	2.8
3	Detritus	3.3	19.8 (3.7)	212 (91)	14
4	Detritus	4.2	13.6 (4.5)	82.4 (20)	9.8
5	Detritus	5.1	26.7 (4.4)	132 (20)	11
6	Detritus	7.0	24.5 (3.8)	150 (60)	5.7
7	Detritus	8.4	56.1 (1.9)	152 (12)	8.7
8	Detritus	10.1	6.16 (1.3)	17.0 (4.8)	22
1	0-10 cm	1.4	5.20 (1.0)	280 (22)	4.4
2	0-10 cm	2.3	8.24 (2.1)	418 (78)	4.1
3	0-10 cm	3.3	8.32 (0.3)	214 (16)	3.3
4	0-10 cm	4.2	4.08 (0.6)	299 (51)	3.7
5	0-10 cm	5.1	35.4 (1.9)	577 (89)	3.0
6	0-10 cm	7.0	6.80 (1.6)	245 (35)	4.1
7	0-10 cm	8.4	27.0 (5.2)	82.2 (15)	3.3
8	0-10 cm	10.1	6.96 (0.2)	244 (50)	4.1
1	10-30 cm	1.4	2.14 (0.79)	183 (28)	10
2	10-30 cm	2.3	1.94 (0.17)	56.9 (20)	12
3	10-30 cm	3.3	1.77 (0.20)	93.9 (14)	11
4	10-30 cm	4.2	2.19 (0.27)	39.2 (16)	12
5	10-30 cm	5.1	3.40 (0.44)	326 (63)	8.5
6	10-30 cm	7.0	1.63 (0.93)	183 (37)	7.5
7	10-30 cm	8.4	2.40 (0.44)	12.4 (29)	14
8	10-30 cm	10.1	1.60 (0.98)	61.3 (23)	14

(Reddy et al, 1989). There was no significant relationship of initial nitrification rates with distance from along the transect (Figure 4-4).

Initial nitrification rates were most strongly correlated with extractable NH_4^+ of soil and detritus (Figure 4-5). This is most likely a coincidental relationship. Organic N mineralization rates are highest in the surface and therefore, the concentration of extractable NH_4^+ is also highest in the surface. Nitrification in these soils was primarily O_2 limited, as seen in the significantly higher potential nitrification rates during the course of the experiment. Therefore, initial nitrification rates were highest in the surface soils, due to the closer proximity and greater availability of O_2. However, the correlation with extractable NH_4^+ could be significant with the initiation of aerobic conditions, linked to substrate availability for nitrification.

Potential rates of nitrification averaged 159 (S.E. = 32), 295 (S.E. = 52), and 119 (S.E. = 37) mg N kg d^{-1} for detritus, 0-10, and 10-30 cm soil depths (Table 4-3). Due to the high variability, there were no significant differences detected in potential nitrification rates between the detritus and 0-10 cm depths, however, there was a significant ($P < 0.05$) difference between nitrification rates for the 0-10 and 10-30 cm depth.

On average, potential rates of nitrification were 13 times greater than the initial nitrification rates (Table 4-3). Of measured soil characteristics, potential rates of nitrification demonstrated a weak correlation ($P < 0.01$; $r = 0.50$) with total P (Table 4-2). The nitrifying populations are autolithotrophs unlike the heterotrophic

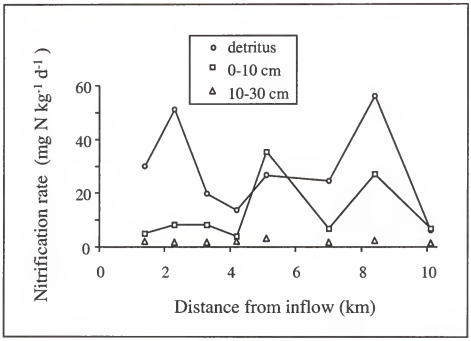

Figure 4-4. Initial nitrification vs distance from inflow for detritus, 0-10 cm, and 10-30 cm soil depths from along the transect in WCA-2A.

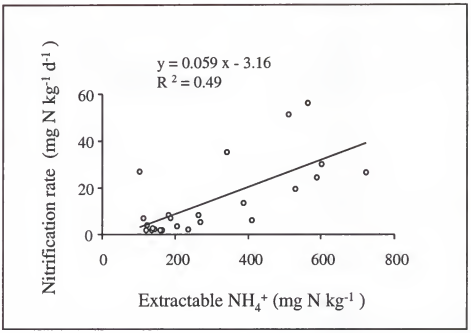

Figure 4-5. Initial nitrification vs extractable NH_4^+ for detritus, 0-10 cm, and 10-30 cm soil depths from along the transect in WCA-2A.

populations involved in N mineralization and denitrification processes. Consequently, the heterotrophic N transformation processes demonstrated significant differences with depth, due to differences in carbon availability which appears to control, to some extent, microbial processes along the transect in WCA-2A (DeBusk, 1996). The autolithotrophs do not require an organic C source from which to derive energy from (electron donor), but instead utilize NH_4^+, which is abundant in these wetland soils. Therefore, the only limit to nitrification, after full aeration, would likely fall to a limiting nutrient to the microbial biomass, in this case, P.

Denitrifying Potential

The potential denitrification rates were highest and not significantly different, in the detritus and 0-10 cm soil depths averaging 13.1 (S.E. = 1.45) and 14.1 mg N kg^{-1} h^{-1} (S.E. = 1.62; Table 4-4). Rates of potential denitrification were significantly lower ($P <$ 0.001), averaging 0.51 (S.E. = 0.07) in the 10-30 cm soil depth and were significantly correlated to distance from inflow in the detrital and 0-10 cm soil depths (Figure 4-6). Denitrification rates were significantly ($P < 0.01$) with MBC, MBN, and extractable NH_4^+ (Table 4-2).

Total P exhibited a significant correlation with potential denitrification at r = 0.85 (Figure 4-7). The denitrification enzyme activity (DEA) of soils was strongly correlated to total P along the transect in WCA-2A, and was further demonstrated to be a coincidental correlation, as NO_3^- was the limiting factor for DEA in the field (see Chapter 5). However, after the 25 d aerobic conditions of the reactor slurries, NO_3^-

Table 4-4. Potential denitrification rates of detritus and soils collected along the transect in WCA-2A during October 1997. Rates were determined in anaerobic bottle incubations after 25 d of aerobic conditions. Data are mean values with one standard error in parentheses.

Station	Distance	Detritus	0-10 cm	10-30 cm
	(km)	---------- mg N kg^{-1} h^{-1} ----------		
1	1.4	17.2 (2.4)	21.6 (2.0)	0.23 (nd)
2	2.3	14.9 (3.2)	17.3 (5.4)	0.41 (0.20)
3	3.3	18.6 (6.3)	17.0 (6.6)	0.71 (nd)
4	4.2	12.2 (0.9)	12.7 (3.0)	0.60 (0.35)
5	5.1	15.5 (1.6)	14.0 (1.4)	0.82 (nd)
6	7.0	9.5 (2.2)	14.2 (2.9)	0.47 (0.25)
7	8.4	6.7 (0.5)	8.35 (1.1)	0.28 (nd)
8	10.1	10.2 (2.0)	7.98 (1.4)	0.53 (0.19)

(nd) = not determined - mean of 2 samples only

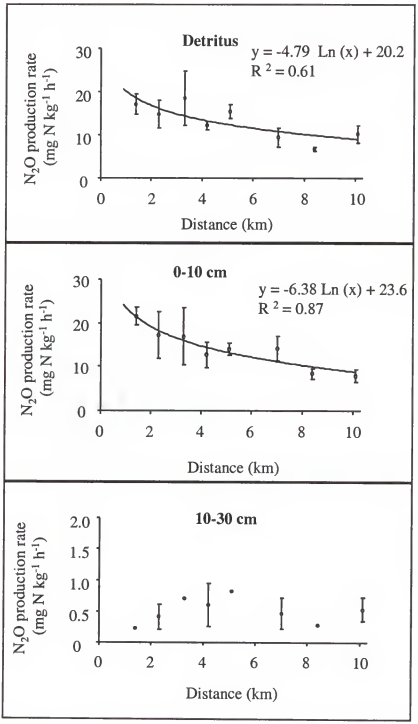

Figure 4-6. Potential denitrification rates of detritus, 0-10 cm, and 10-30 cm soil depths from along the transect in WCA-2A. Plotted are means and one standard error.

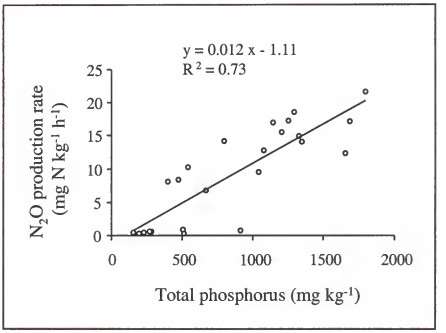

Figure 4-7. Potential denitrification rate vs total P for detritus, 0-10 cm, and 10-30 cm soil depths from along the transect in WCA-2A after 25 d of aerobic conditions.

concentrations were large, due to nitrification of the initial extractable NH_4^+ and the additional NH_4^+ liberated from the organic fraction under relatively high aerobic N mineralization rates. Consequently, NO_3^- was no longer a limiting factor in any of the soil treatments and it is likely another nutrient was limiting to denitrification under these high NO_3^- conditions. Phosphorus was likely limiting denitrification rates at these relatively high NO_3^- levels.

The facultative nature of the microbial denitrifiers was clearly exhibited in the coupled nitrification-denitrification study. The soil samples within the reactors were completely aerobic, due to continual mixing and aeration with room air for ~ 25 d. Samples placed under an anaerobic headspace with acetylene, very quickly (within 12 h) began producing substantial quantities of N_2O. It is not surprising that these facultative anaerobic bacteria are concentrated in the surface of wetland soils, which can periodically switch from aerobic to anaerobic systems daily. This allows the faculatative anaerobes a distinct competitive advantage in this particular ecological niche, as these bacteria appear to be able to switch off respiratory systems in very short order. This is also the very reason that wetland treatment systems, either designed or natural, are very effective at reducing wastewater NO_3^- levels.

Conclusions

Total P was significantly correlated with depth and distance from the surface water inflow point along transect in WCA-2A, demonstrating that continual P loading

over the past 35-40 y has resulted in a soil total P gradient. Microbial biomass C and N were significantly higher in the surface soils and were correlated to the extractable NH_4^+ concentration. Initial nitrification rates were low, likely limited by a low, active microbial population present in the soil due to the low O_2 and high SOD in these organic soils. Potential nitrification rates were, on average, 11 times greater than initial rates. Potential denitrification rates appeared to be limited by the total P content of the soil and decreased by first order decay with increasing distance from the inflow. Results suggest that O_2 availability to the soil is the greatest limiting factor for nitrification in WCA-2A. Consequently, this limitation on nitrification controls the rate at which NH_4^+ is removed from the wetland by the coupled processes of nitrification-denitrification.

These results have significant consequences for the management of the WCA-2A by the SFWMD. The presence and depth of the water column is critical in maintaining a stable N balance in the ecosystem, as aerobic soil conditions would significantly increase the rate of inorganic N removal out of the system into the atmosphere by coupled nitrification-denitrification. Soils from within the impacted region closest to the surface water inflow point demonstrated highest denitrifying potential. These higher rates appear to be a consequence of increased soil total P, which likely limits the microbial pool in the natural marsh areas located in the interior of WCA-2A.

CHAPTER 5
INFLUENCE OF NITRATE AND PHOSPHORUS LOADING ON DENITRIFYING ENZYME ACTIVITY

Introduction

Nitrate reduction is the major N removal mechanism in wetlands. Among NO_3^- reduction processes, denitrification is the dominant NO_3^- removal process. Denitrification is a microbial mediated process whereby facultative anaerobic bacteria use NO_3^- (or NO_2^-) in the absence of O_2 as the terminal electron acceptor during the oxidation of organic C (microbial respiration) resulting in the production of gaseous end products, N_2O and N_2. The denitrification enzyme assay (DEA) is used as a means to eliminate all other regulating factors of denitrification in order to quantify the amount of active denitrifying enzymes present in soil (Smith and Tiedje, 1979; Smith and Parsons, 1985; Groffman, 1987; Martin et al., 1988; Parsons et al., 1991; Schipper et al., 1993). The enzyme assay is the short term (2 h) rate of NO_3^- reduction, and is indicative of the size and activity of the extracellular denitrifying enzyme pool present in soil and reflects the immediate biological effect of changes in redox conditions attributed to changes in soil O_2 levels (Martin et al., 1988).

Several studies of DEA have focused on upland soils (Smith and Tiedje, 1979; Groffman, 1987; Martin et al., 1988; Parsons et al., 1991) with the goal of more recent studies focused on determining denitrification potential at the ecosystem scale, correlated

to easily measurable soil parameters. These studies have reported rates of N_2O production in upland soils ranging from 0.006 to 7.14 mg N kg^{-1} h^{-1}. The results of such research could be used to quantify the contribution of soils on the landscape to global atmospheric N_2O levels.

Several problems associated with the use of DEA in extrapolating to landscape scale denitrification rates in upland soils exist. The microorganisms responsible for the production of enzymes are facultative anaerobes, which possess separate enzyme systems capable of utilizing either O_2 or NO_3^- as terminal electron acceptors. The NO_3^- reducing enzyme systems are primarily inactive in the presence of O_2, and active in enzyme production only during ephemeral anoxic events (eg. rainfall events; Sexstone et al., 1985; Burton and Beauchamp, 1985). Further, the presence of O_2 appears to repress or de-activate enzymes already present in soil (Martin et al, 1988). Secondly, the distribution of organic matter in upland systems is patchy which presents additional problems in assessing the spatial distribution of DEA. Hotspots of organic matter provide both simple C compounds for the maintenance of large, microbial populations and anoxic micro-environments which lead to NO_3^- consumption as the terminal electron acceptor (Parkin, 1987; Christiansen et al., 1990a, b). The patchy distribution of active enzyme sites in the landscape leads to logarithmic frequency distributions of enzyme activity measured in the field (Parkin, 1987). Finally, NO_3^- is rarely the limiting factor for denitrification in upland ecosystems, evidenced by the widespread problem of groundwater contamination of NO_3^-, and consequently is a poor indicator of denitrification potential. These factors in combination, confound efforts to consistently correlate easily measured soil parameters (total C, water content, NO_3^- concentration and microbial biomass) with DEA to produce meaningful estimates of denitrification in the

landscape. (Velthof, 1996; Martin et al., 1988; Parsons et al., 1991).

There exist several important differences between mineral, upland soils and organic-rich, wetland soils that permit the reliable use of DEA on a landscape scale in order to investigate source and effect of NO_3^- loading in wetlands. Wetland soils are often saturated most of the year, thereby reducing diffusion of O_2, resulting into all but the smallest (1-4 mm) surface layer of soil remaining anaerobic (DeBusk and Reddy, 1998). Christensen et al. (1990b) found that the frequency distribution of denitrification rates was less skewed in upland soils after the onset of flooded conditions. This paucity of O_2 prevents both the repression of enzymes present and halts production of new enzymes by the facultative anaerobes. The relatively high organic matter content of wetland soils provide ample substrate for heterotrophic microbial activity and any O_2 which contacts the soil is quickly utilized. These conditions suggest that NO_3^- supply is the limiting factor for denitrification in wetlands (Cooper, 1990), and the presence of NO_3^- will therefore control the size and activity of the denitrifier microbial populations. Schipper et al. (1993) found that up to 77% of the variability of *in situ* denitrification in an organic riparian wetland soil could be explained by NO_3^- concentration and DEA. They also noted that organic soils were responsible for 56 – 100 % of total NO_3^- consumption despite comprising only 12% of total soils in the catchment.

In an oligotrophic wetland, there should exists a characteristic baseline level of DEA in the soil, as NO_3^- is supplied to the microbial pool through nitrification, occurring in the water column and in the aerobic zones of the soil (Reddy and Patrick, 1984). The DEA levels above site-specific baseline values should provide strong evidence of an allochthonous source of NO_3^-. Therefore, the spatial distribution of DEA might be used as an effective tool in (1) identifying sources of NO_3^- pollution to wetlands, (2)

elucidating the areal extent of influence of NO_3^- pollution, and (3) calculating potential removal rates of NO_3^- on the landscape scale.

Study Area

The Florida Everglades are currently affected by nutrient loading from urban and agricultural surface runoff. Most notably, this impact is seen in the Water Conservation Areas, one of the major hydrologic units of the Everglades (DeBusk et al, 1994). Water Conservation Area 2A has been receiving nutrient-laden (N and P) drainage waters for the past 30 years. Peat and nutrient (organic C, N and P) accretion rates have increased in areas receiving surface drainage water (Koch and Reddy, 1992; Craft and Richardson 1993). Most notably, the impact of anthropogenic nutrient loading is documented in the spatial distribution of surface soil total P. Total P concentration grades from a high of ~1600 mg kg^{-1} at the surface water inflow points to a background concentration of ~400 mg kg^{-1} in unimpacted areas located in the interior of the marsh (Koch and Reddy, 1992; Reddy et al., 1993; DeBusk et al., 1994). A gradient in N and P concentrations in the water column and periphyton tissue has also been documented along the same transect in WCA-2A (McCormick and O'Dell, 1996). Historically an oligotrophic, P-limited sawgrass (*Cladium jamaicense* Crantz) marsh, the vegetation began a shift towards a dominant cattail (*Typha domingensis* Pers.) vegetative community proximal to all surface water inflow points (Davis, 1991; Craft and Richardson, 1997). The timing of vegetative shift coincided with the initiation of diversion of surface water pumped primarily from the canal network draining the Everglades Agricultural Area (EAA) approximately for the past 40 years.

Objectives

The objectives of this study were to (1) determine the spatial and temporal distribution of DEA for wetland soils of WCA-2A, (2) determine the effects of added P and NO_3-N on DEA, and (3) investigate selected soil parameters as indicators of DEA.

Materials and Methods

Experimental Design

Ten stations were located along a 10 km transect originating from the S-10C inflow water control structure in WCA-2A (Figure 5-1). The study transect spanned the marsh from a primary water control inflow structure (S-10C), southward across the dominant cattail (*Typha sp.)* vegetation and terminating ~ 10 km into the natural (unimpacted) marsh characterized by stunted stands of sawgrass (*Cladium* sp.) separated by shallow sloughs dominated by floating and attached cyanobacterial mats. Sampling stations were located at distances of 0.2, 0.3, 1.4, 2.3, 3.3, 4.2, 5.1, 7.0, 8.4 and 10.1 km from the S-10C water control structure. Water depths varied seasonally from <2 cm to ~ 2 m along the transect length.

Sampling along the transect was not designed to identify differences between individual stations, but rather to investigate gradients or trends in soil characteristics, including DEA, with distance. Soils were collected four times over a two year span (August 1995, February and August 1996, and March 1997). Sampling times were

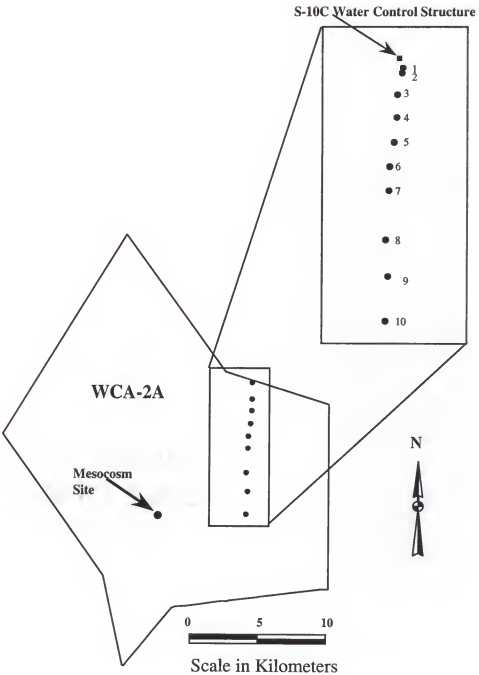

Figure 5-1. Station locations along soil phosphorous gradient in WCA-2A (south of S-10C water control structure) used in the study.

selected to best assess the effect of changes in hydraulic loading rates of surface waters on denitrification rates, related to both the wet season (summer) and the dry season (winter).

The South Florida Water Management District (SFWMD) established 21 circular tanks (mesocosms) enclosing 1.8 m^2 and three open control plots in an unimpacted sawgrass-periphyton-slough (McCormick and O'Dell, 1996). The mesocosm site was located approximately 11 km SW from the S-10C inflow water control structure (Figure 5-1). The mesocosms were installed entirely within a shallow slough which contained no established stands of sawgrass within the study site. The soil surface was dominated by floating and benthic cyanobacterial (periphyton) mats, purple bladderwort (*Utricularia purpurea* Walt.) and water lily (*Nymphaea odorata* Ait.) (McCormick and O'Dell, 1996). Three replicate mesocosms were selected at random and spiked once a week with various amounts of NaH_2PO_4 mixed with slough water to achieve annual loading of 0, 0.4, 0.8, 1.6, 3.2, 6.4 and 12.8 g P m^{-2} yr^{-1}. The tanks were closed from exchange with the surrounding water by sliding a plastic collars over the holes for 24 h after spiking. The tanks were subsequently opened to permit exchange with the surrounding slough during the no dose periods. Prior to our soil sampling, these systems had been dosed weekly at respective levels for a period of 17 months.

Soil Characterization

A minimum of four soil cores was collected within 5 m of each station along the transect by driving a 10 cm diameter aluminum irrigation pipe into the soil. A probe was inserted into each core to verify that negligible (<5%) compaction had occurred during coring. Cores were sealed, removed from the ground, immediately extruded and

separated into separate soil intervals (0-10 and 10-30 cm) in the field. Each interval was well mixed to yield a representative and homogenous sample from each depth at each station. The August 1995 and February 1996 samples were bagged and immediately transported to the laboratory in Gainesville on ice. Samples were transferred into 2 L polyethylene containers within 24 hr of collection and stored refrigerated at 4°C until analysis. Soil samples collected in August 1996 and March 1997 were immediately transported to a field "laboratory" location and incubated within 3 h of collection. Detrital surficial litter material was collected during the last two sampling events for use in field incubations. Detritus consisted of recognizable, loosely associated cattail or sawgrass plant material lying on the surface of the underlying more compact, brown, peat soil. The detrital layer varied in thickness from <1 cm in sawgrass areas to >25 cm at the cattail stations closest to the inflow. The remaining soil samples not utilized in field incubations, were sealed in plastic bags and kept on ice until return to the laboratory where the samples were transferred into polyethylene containers and refrigerated at 4°C until subsequent characterization.

Soils were collected from experimental mesocosms on November 21, 1996 by driving a 10 cm diameter polyethylene tube into the soil. The periphyton-floc layer was poured off into separate sampling containers. The top soil interval (0-3 cm) was then extruded, stored in plastic bags and placed on ice until returning to the laboratory where samples were stored refrigerated at 4°C until subsequent characterization.

Bulk density was calculated for the soil intervals on a dry weight basis. Total C and N contents of detritus and soils were determined on dried, ground samples using a Carlo-Erba NA-1500 CNS Analyzer (Haak-Buchler Instruments, Saddlebrook, NJ). Total P analysis was performed on sub-samples prepared by nitric-perchloric acid digestion

(Kuo, 1996) and analyzed using an automated ascorbic acid method (Method 365.4, USEPA, 1983).

Microbial Biomass

Microbial biomass C (MBC) was determined using the fumigation-extraction technique of Vance et al. (1987) for the February and August 1996 and March 1997 sampling times. Six replicate 5 g samples were placed into 25 ml centrifuge tubes for each soil interval and all eight sampling sites. One half ml of chloroform was added to three replicate tubes and placed in a vacuum desiccator with a beaker containing 300 ml of chloroform and several boiling chips.

After 24 h, the headspace was alternatively evacuated and filled with room air nine times to remove chloroform still present in the soil or beaker. Samples were removed and both the controls (not exposed to chloroform) and chloroform treated soils were immediately extracted with 20 ml of 0.5 *M* K_2SO_4, agitated for 30 min on a longitudinal shaker and vacuum filtered through #42 Whatman filter paper. The supernatant was collected and refrigerated at 4°C until analyzed on a Dohrman TOC analyzer. Microbial biomass was determined by subtracting the extractable total organic carbon (TOC) in the triplicate controls from the triplicate chloroform-treated samples. An extraction efficiency (k_{EC}) factor of 0.37 was applied, utilizing a previous calibration for organic soils by Sparling et al. (1990).

Denitrifying Enzyme Activity

Laboratory DEA incubations were performed on soils collected along the transect in August 1995 and February 1996 and on soils collected from the mesocosms in November 1996. Approximately 10 g of field moist soil from each site and depth were placed in triplicate 110 ml glass serum bottles and sealed with rubber septa and aluminum crimp caps. The headspace was evacuated to -85 kPascals and replaced with O_2-free N_2 gas to achieve anaerobic conditions. Five ml of N_2-purged distilled-deionized water were added to each bottle to create a soil slurry. Approximately 15 % of the headspace N_2 was replaced with acetylene gas (C_2H_2) (Balderston et al., 1976, Yoshinari and Knowles, 1976). Bottles were shaken on a longitudinal shaker for 1 h to evenly distribute the C_2H_2 throughout the soil slurry. Eight ml of solution, containing 56 mg NO_3-N L^{-1}, 288 mg $C_6H_{12}O_6$-C L^{-1} and 2 mg L^{-1} chloramphenicol (an enzyme production inhibitor) were added to each bottle, creating a slight overpressure (Smith and Tiedje, 1979). Samples were incubated in the dark at 25°C and continually agitated on a longitudinal shaker. Headspace gas was sampled every 30 min over a 2 h period. Nitrous oxide production was adjusted for N_2O dissolved in the aqueous phase using Bunsen absorption coefficients (Tiedje, 1982). The denitrification rate was calculated by determining the slope of the linear curve produced when cumulative N_2O evolution was plotted vs time.

Field DEA incubations were performed on freshly collected soils (during August 1996 and March 1997) within 3 h of sampling. Incubations followed the same procedures as those performed in the laboratory, with the following modifications due to field constraints; bottles were evacuated by pulling a 60 cm^3 syringe three times to evacuate the headspace and incubated submerged in site water at ambient temperatures (~ 29-31°

C) without shaking; headspace gas was sampled at the terminus of the 2 h incubation, placed in evacuated 3.5 ml serum bottles sealed with butyl rubber stoppers and aluminum crimp caps and transported to the lab for subsequent gas analysis within 48 h.

Potential Denitrification Rates

Surface soils (0-10 cm) from Station 1 (located 0.2 km from the water control structure) and Station 10 (located 10.1 km from water control structure) were subjected to inflow water concentrations of NO_3^- (~ 1 mg L^{-1} NO_3-N) under a 15 % C_2H_2 headspace for 24 h without addition of an enzyme inhibitor or exogenous carbon in order to determine the denitrification potential of soils from outside the impacted region in response to increased NO_3^- loading. Samples were continuously shaken at 25°C in the dark to negate diffusion limitations. Headspace gas was sampled periodically until the N_2O production curve flattened out, indicating the complete consumption of added NO_3^-. The potential denitrification rate at this inflow floodwater NO_3^- concentration was calculated from the steepest part of the N_2O production curve.

Nutrient Addition Study

Surface soil (0-10 cm) from Station 10 (10.1 km from the inflow) was collected to determine the effect of added $NaNO_3$-N and $NaHPO_4$-P on DEA. The soil was homogenized by mechanical mixing after removing live roots. Approximately 50 g of field moist soil was added to each 120 ml media bottle equipped with a butyl rubber septum embedded in the cap. To each bottle, 40 ml of distilled deionized water was

added and mixed well with the soil. The following treatments were evaluated; (1) control-no additions, (2) NO_3-N added (1, 10, 50, 100 mg L^{-1} porewater concentration), (3) PO_4-P added (0.1, 1, 5, 10 mg L^{-1} porewater concentration), and (4) NO_3-N + PO_4-P added (matching rates as shown in treatments 2 and 3, with the lowest rate of 2 added in conjunction with the lowest rate in 3, etc). Each treatment was performed in triplicate. Bottles were capped and purged with O_2-free N_2 gas to induce anaerobic conditions. Samples were incubated in the dark at 30°C for 10 d and were shaken by hand for 30 sec d^{-1}. Bottles were then re-spiked with the same concentration of the respective solutions and incubated for an additional 10 d. A triplicate set of soil controls was spiked with distilled deionized water and was included in the incubation. At the terminus of 20 d incubation, sample bottles were opened and 20 ml of slurry was collected from each bottle, placed in a serum bottle under a N_2 headspace containing 15% C_2H_2. Potential denitrification rates were determined without NO_3^- additions over 48 h for all samples. An additional 20 ml of soil slurry was subjected to the denitrification enzyme assay procedure to investigate the effects of nutrient additions on soil DEA values.

Gas Analysis

Gas samples were analyzed for N_2O on a Shimadzu gas chromatograph (GC-14A) equipped with a 10 mCi ^{63}Ni electron capture detector (ECD). A 1.8 m x 2mm ID stainless steel column packed with poropak Q (80-100 mesh) was used. The carrier gas (5% methane in argon) flow rate was 30 ml min^{-1}. Working standards consisted of N_2O in a framework of He gas (Scott Specialty Gas, Inc.; Plumsteadville, PA).

Data Analysis

Data were fitted to an analysis of variance (ANOVA) model to investigate significant differences ($P < 0.05$) in DEA among stations, soil depths, seasons, and nutrient addition levels. A paired students t-test was used to detect significant differences ($P < 0.05$) between seasonal distributions of DEA along the transect length. DEA also was statistically correlated with soil characterization data to determine which soil parameters were the best predictor(s) of enzyme activity. Fisher's Least Significant Difference (LSD) test was utilized to make comparisons among treatment levels for the nutrient addition study, using the StatGraphics software program (Manugistics, Inc., Rockville, MD).

Results and Discussion

Soil Characterization

Average bulk densities of organic soils collected were 0.064 and 0.089 g cm^{-3} for the 0-10 cm and 10-30 cm soil depths, respectively (Table 5-1). Bulk densities were not determined for detritus samples. Total C and N did not vary significantly along the transect, but total P was significantly negatively correlated ($P < 0.01$) with distance from the inflow for detritus, 0-10 cm and 10-30 cm soil depths (r = -0.882, -0.971 and –0.833, respectively). Results of a one-way ANOVA showed that total P was significantly higher ($P < 0.05$) in both detritus and 0-10 cm soil when compared with the underlying 10-30 cm soil while there was no significant difference in total P content of detritus and the 0-

Table 5-1. Selected physiochemical properties of detritus and soils collected from along the study transect in WCA-2A. Data are mean values (n=3) with 1 standard deviation in parentheses (data from stations at 0.2 and 0.3 km are means of n=2).

Distance km	Depth	Bulk Density g cm-3	Total C g kg^{-1}	Total N g kg^{-1}	Total P mg kg^{-1}
0.2	detritus	n.d.	418	24.8	1582
0.3	detritus	n.d.	452	25.3	1461
1.4	detritus	n.d.	450 (24.1)	25.9 (2.8)	1601 (287)
2.3	detritus	n.d.	432 (10.4)	25.8 (1.4)	1615 (177)
3.3	detritus	n.d.	443 (20.2)	22.0 (3.6)	1509 (122)
4.2	detritus	n.d.	431 (19.5)	21.0 (3.1)	1456 (459)
5.1	detritus	n.d.	434. (16.6)	20.7 (2.0)	1096 (274)
7.0	detritus	n.d.	414 (47.8)	21.8 (4.8)	829 (256)
8.4	detritus	n.d.	403 (24.0)	24.8 (4.2)	556 (177)
10.1	detritus	n.d.	419. (7.6)	22.9 (3.5)	417 (25)
0.2	0-10 cm	0.086	392	27.5	1497
0.3	0-10 cm	0.086	380	25.1	1337
1.4	0-10 cm	0.049 (0.0062)	400 (20)	27.0 (2.3)	1552 (102)
2.3	0-10 cm	0.050 (0.016)	440 (14)	28.2 (2.5)	1369 (100)
3.3	0-10 cm	0.052 (0.011)	455 (33)	29.6 (4.1)	1205 (105)
4.2	0-10 cm	0.070 (0.015)	449 (40)	29.0 (3.4)	989 (144)
5.1	0-10 cm	0.067 (0.011)	433 (15)	27.4 (3.0)	966 (61)
7.0	0-10 cm	0.06 (0.006)	424 (34)	30.0 (2.2)	641 (152)
8.4	0-10 cm	0.067 (0.005)	445 (31)	29.3 (2.2)	486 (113)
10.1	0-10 cm	0.06 (0.002)	440 (23)	28.4 (4.0)	479 (124)
0.2	10-30 cm	0.088	385	27	1195
0.3	10-30 cm	0.059	441	28.7	1172
1.4	10-30 cm	0.086 (0.018)	444 (5.6)	32.0 (0.4)	570 (98)
2.3	10-30 cm	0.104 (0.011)	464 (9.8)	30.1 (2.7)	373 (10)
3.3	10-30 cm	0.094 (0.003)	474 (27)	33.8 (5.5)	363 (68)
4.2	10-30 cm	0.092 (0.006)	469 (31)	31.8 (4.9)	287 (38)
5.1	10-30 cm	0.074 (0.012)	395 (113)	26.4 (5.6)	289 (31)
7.0	10-30 cm	0.098 (0.004)	427 (35)	25.2 (4.0)	247 (84)
8.4	10-30 cm	0.088 (0.0006)	478 (26)	28.6 (3.9)	229 (14)
10.1	10-30 cm	0.074 (0.009)	474 (21)	30.2 (3.9)	246 (32)

n.d. = not determined

10 cm soil depth. Microbial biomass C (MBC) was significantly negatively correlated with distance ($r = -0.68$; Table 5-2). A positive correlation was observed for MBC vs total P ($r = 0.65$) for the detrital layer. The MBC decreased with depth along the transect ($P < 0.01$; $r = 0.69$). Microbial biomass C averaged 13.3, 4.8, and 1.6 g kg^{-1} for the detritus, 0-10 cm, and 10-30 cm soil depths respectively.

Soil bulk density from the mesocosms did not vary significantly between treatments ($P > 0.9$) and averaged 0.092 g cm^3 for the 0-3 cm soil depth (Table 5-3). Total C and N also did not vary significantly between treatments. Total P was significantly positively correlated ($P < 0.01$) with P-loading rate (Table 5-4). Microbial biomass C was weakly positively correlated with soil total P ($r = 0.50$).

Spatial Distribution of DEA along the Transect

Laboratory results of DEA for the August 1995 and February 1996 sampling dates are shown in Figure 5-2. Results of a single factor ANOVA suggested a significant difference ($P < 0.05$) in DEA between the 0-10 cm and 10-30 cm depths. The DEA was significantly higher in the surface soil averaging 2.69 mg N_2O-N kg^{-1} h^{-1} compared to 0.74 mg N_2O-N kg^{-1} h^{-1} ($P < 0.05$) for the underlying soil (10-30 cm) during the August 1995 sampling. This trend was consistent with the February 1996 sampling with mean values of DEA for 0-10 cm of 1.08 mg N_2O-N kg^{-1} h^{-1} and 0.10 mg N_2O-N kg^{-1} h^{-1} for 10-30 cm at a significance level of $P < 0.05$ (Figure 5-3).

The vertical distribution of DEA for the field studies conducted in August 1996 and March 1997 also included the collection of surficial litter or detritus. There existed

Table 5-2. Pearson product-moment correlation coefficients for selected parameters for soil samples collected from along the transect in WCA-2A. For n = 72, $P < 0.05$ for r = 0.23 and $P < 0.01$ for r = 0.30.

	Distance	DEA	total C	total N	total P	MBC
DEA	-0.30					
total C	-0.04	-0.16				
total N	-0.10	-0.44	0.51			
total P	-0.59	0.73	-0.19	-0.25		
MBC	-0.25	0.77	-0.20	-0.50	0.70	
Depth	0.00	0.62	-0.29	-0.51	0.68	0.69

Table 5-3. Selected physiochemical properties of the 0-3 cm soil depth from the mesocosm experiment in WCA-2A. Data are mean values (n = 3) with 1 standard deviation in parentheses.

P-loading rate mg P m^{-2} y^{-1}	Bulk Density g cm^{-3}	total C g kg^{-1}	total N g kg^{-1}	total P mg kg^{-1}
0 (oc)	0.081 (0.008)	392 (9.3)	30.2 (3.1)	492 (56)
0	0.094 (0.024)	404 (25)	31.8 (4.6)	427 (41)
0.4	0.113 (0.023)	345 (65)	26.6 (6.5)	475 (65)
0.8	0.101 (0.032)	349 (11)	26.4 (2.0)	471 (71)
1.6	0.074 (0.015)	424 (4.2)	32.9 (0.5)	380 (55)
3.2	0.096 (0.033)	366 (48)	25.4 (2.2)	460 (34)
6.4	0.093 (0.011)	383 (26)	29.5 (3.8)	594 (40)
12.8	0.093 (0.034)	355 (62)	27.6 (7.6)	688 (240)

(oc) = open control

Table 5-4. Pearson product-moment correlation coefficients for selected parameters for soil samples collected from the mesocosm experiment in WCA-2A. For n = 24 at $P < 0.05$; r = 0.40 and at $P < 0.01$; r = 0.52.

	DEA	P-Loading	total C	total N	total P
P-Loading	0.04				
total C	0.25	-0.18			
total N	0.18	-0.14	0.86		
total P	0.07	0.69	-0.24	-0.31	
MBC	0.00	0.32	-0.36	-0.34	0.50

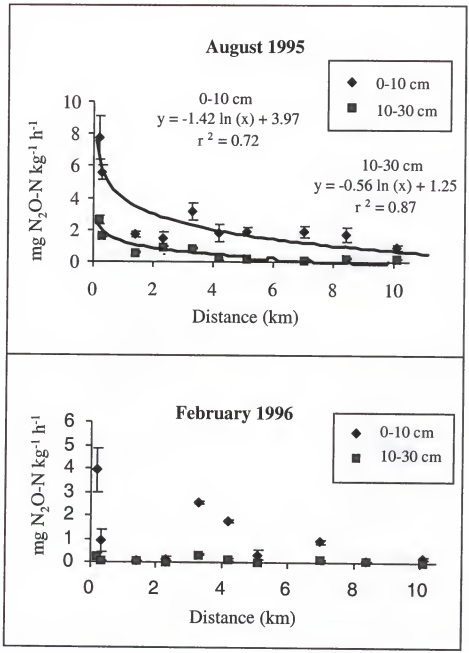

Figure 5-2. Plot of DEA vs distance for 0-10 and 10-30 cm soil interval for the August 1996 and February 1997 sampling from along the study transect in WCA-2A.

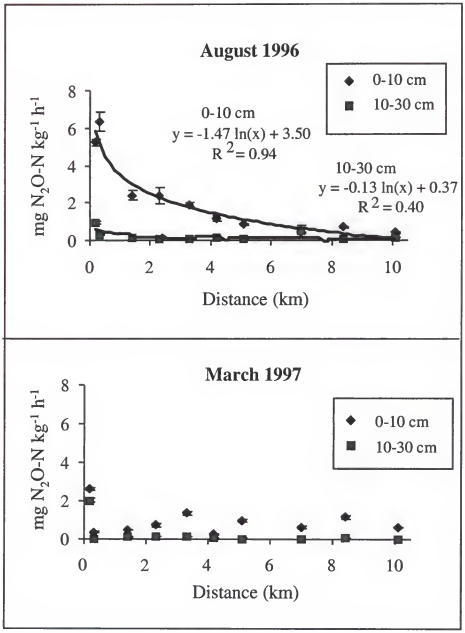

Figure 5-3. Plot of DEA vs distance for 0-10 and 10-30 cm soil interval for the August 1996 and March 1997 sampling from along the study transect in WCA-2A.

no significant difference between detritus and the 0-10 cm depth for the August 1996 sampling, but a highly significant difference ($P < 0.01$) for both detritus and 0-10 cm vs the 10-30 cm depth.

The results of a 2-way ANOVA for all 4 samplings (distance x sampling time) demonstrated a significant difference ($P < 0.01$) along the transect for the 0-10 cm soil depth, with highest rates closest to the inflow. Data for surface (0-10 cm) soil DEA from both August 1995 and 1996 significantly fit an exponential decay model fit ($R^2 = 0.72$ and 0.94, respectively) suggesting continual NO_3^- loading during the previous months and subsequent loss of NO_3^-. The exponential model appeared to flatten out below 2 mg N_2O-N kg^{-1} h^{-1} at a distance of ~2 km from the inflow. The significant decrease in denitrification and DEA with increasing distance from inflow in wetlands is consistent with results reported by Gale et al. (1993) for a constructed wetland receiving NO_3^-. The authors found denitrification rates up to 10 times higher at a stations proximal to the inflow of a wetland receiving reclaimed wastewater (0.6 mg L^{-1} of NO_3-N) than at a station located 300 m further into the marsh.

Denitrifying enzyme activity of detritus and surface soils within 2 km of the inflow in WCA-2A are among the highest rates reported in the literature (Table 5-5), and are attributed to the stimulation of denitrifying bacteria by the inflow concentrations of NO_3^- in agricultural drainage waters. Lower levels of DEA at stations furthest from the inflow are likely related to nitrification processes in the overlying water column, producing NO_3^- from NH_4^+ which then diffuses into the soil under a concentration gradient where it can be utilized by denitrifiers (Reddy and Patrick, 1984).

Stations 3 and 4, located 1.4 and 2.3 km from the inflow respectively, contained surface soil DEA values less than 1 mg N_2O-N kg^{-1} h^{-1} for the August 1995 sampling

Table 5-5. Literature values for DEA as determined for upland and riparian systems.

Location	Soil Use/Type	DEA	Reference
Kentucky	Mollisol Upland under sod	0.93 ± 0.05	Martin et al. (1988)
Kentucky	Agricultural Mollisol, Alfisol	0.006 - 7.14	Parsons et al. (1991)
Kentucky	Permanent Pasture Alfisol	0.102 - 5.4	Smith and Parsons (1985)
Upsalla Sweden	Agricultural Sandy clay	0.33 - 1.74	Pell et al. (1996)
Devon, UK	Agricultural Silty clay	1.25 - 1.87	Dendooven and Anderson (1995)
North Island, New Zealand	Riparian Inceptisol	0.81 ± 0.4	Schipper et al. (1993)
North-central Florida	Dairy (riparian) Entisol	0.013 - 0.03	Yan (1995)
Okeechobee, Florida	Agricultural Spodosol	0.13 ± 0.05	Espinoza (1997)
Everglades, Florida	Wetland Histosol	0.04 - 7.75	This study

(see Figure 5-2) and also recorded low rates during the February 1996 sampling. It is possible this area is somewhat hydraulically isolated, due to the extreme thickness of cattail (*Typha* sp.) and willow (*Salis* sp.) which can retard the movement of inflow surface waters. The DEA values appeared to increase at 3 km, leading to speculation that groundwater might be entering the wetland at this point carrying NO_3^-. It is unlikely that the increase in DEA at 3 km is due to groundwater flow containing NO_3^-, however, as the underlying soils (10-30 cm) also contain low DEA. Denitrifying enzyme activity in soils from 10-30 cm depth can increase to comparable rates found for surface soils spiked with NO_3^- during laboratory incubations. Therefore, it is likely that NO_3^- is limiting at stations 3 and 4 due to hydraulic isolation. This explanation is further supported by high levels of DEA observed for both detritus and surface soils at these stations (3 and 4) during the August 1996 field study (Figure 5-3). The surface water in the marsh was deep (>50 cm), perhaps increasing surface hydraulic conductivity along the transect at the time of sampling. The increased water depth allowed the detritus to float, increasing the hydraulic conductivity of surface water containing NO_3^- through the detrital mat, and could have led to increased DEA values.

Temporal Distribution of DEA

The importance of seasonal trends is linked primarily to differences in precipitation and the consequent need for surface water management during the wet (summer) season in this region. The increased diversion of surface waters into the WCAs during the summer months would consistently lead to greater nutrient loading of both N and P.

The results of a paired student's t-test suggest higher DEA for the summer or wet season (August 1995) 0-10 cm soil data compared with the winter or dry period (February 1996), at $P < 0.05$. A similar trend was evident in the 10-30 cm soil depth at $P < 0.05$, with higher DEA in August 1995 than in February 1996. Highest DEA was found closest to the surface water inflow point during the summer months, further supporting the hypothesis that increased soil DEA is stimulated by NO_3^- loading. There was no significant difference between sampling times for the 10-30 cm soil interval suggesting NO_3^- supply to the subsurface soils is supplied primarily through nitrification processes at the soil/root interface as opposed to diffusion of NO_3^- down from the water column.

The seasonal differences in DEA appear to be related to the surface water management schedule dictated by seasonal differences in rainfall. The SFWMD maintains increased surface water flow into the WCA-2A during the wet summer periods. Both studies in summer (August 1995, 1996) yielded higher N_2O production rates at stations closest to the inflow as compared to the same sites during the winter (February 1996, March 1997). As the majority of NO_3^- loading occurs in the summer (wet season) months, there appears to be a concomitant increase in sustainable numbers of the microbial populations responsible for producing the denitrifying enzymes, utilizing available NO_3^- as an electron acceptor.

Impact of Nitrate Loading on DEA

The areal extent of relatively high DEA values appears to be confined within 2 km of the inflow point, with DEA not significantly changing along the transect after that point. Average yearly NO_3^- removal (denitrification) rate of both soil layers within the

impacted region was calculated to be ~ 2.22 x 10^6 g N d^{-1} in 1995. This total potential NO_3^- removal was integrated over the entire sampled soil volume within the impacted region. The yearly loading rate of NO_3^- to WCA-2A through the S-10C water control structure as determined by the SFWMD in 1995 was 2.50 x 10^6 g N d^{-1}. The strong agreement between these two estimates suggests that all of the NO_3^- loaded to the system could potentially be removed by denitrification within the zone of elevated DEA levels.

Potential denitrification rates are generally an over-estimation of field or *in situ* rates in uplands, as diffusion constraints are present in the field. Shipper et al. (1993), however, found that DEA and field rates of ambient denitrification were similar in an organic riparian wetland. However, hydraulic loading to WCA-2A is sporadic or discontinuous, so it is likely that a plume of inflow water could extend beyond the defined impacted region during high surface-water flow events.

Selected soil samples were subjected to inflow concentrations of NO_3^- (~ 1 mg N L^{-1}), to determine the potential increase in denitrification rate for soils from outside the impact region in response to increased NO_3^- loading. The soil from within the impacted region reduced the added NO_3^- to N_2O gas within 4.5 h of the start of the incubation (Figure 5-4). Denitrification rate was linear and approximated the short-term DEA rate. The "unimpacted" soil demonstrated low initial denitrification rate similar to that for the 2 h enzyme assay. However, stimulated by the added NO_3^- over 10 h, the denitrifier population increased in size and/or activity and produced greater quantities of denitrifying enzymes, increasing the N_2O production rate to the same order of magnitude as the impacted site. This result demonstrates that soils from outside the impacted area are capable of quickly responding to increased NO_3^- loading by increasing biological denitrification rates. Cooper (1990) reported high DEA along the upslope edge receiving

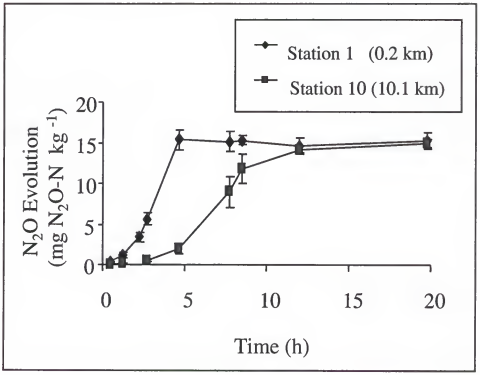

Figure 5-4. Cumulative N_2O production curves for 0-10 cm soil interval located 0.2 km (impacted) and 10.1 km (unimpacted) from the water control inflow structure in WCA-2A. Plotted are means (n=4) and standard error. Samples were collected in March 1997.

NO_3^- inputs to an organic riparian wetland, yet low or undetectable levels of DEA further downslope. The author concluded that the organic soils downslope were capable of higher denitrification, but were simply NO_3^- limited. A similar conclusion was reached by Schipper et al. (1993).

Impact of Phosphorus Loading on DEA

The Everglades is a P limited system (Davis, 1991) and as such, we examined the relationship between soil total P values and DEA. Microbial populations have been found to be limited by soil P in some ecosystems (Wardle, 1992). There existed a highly significant correlation ($r = 0.93$, $P < 0.01$) between total P and DEA for detritus and for the 0-10 cm and 10-30 cm soil depths (Figure 5-5). We thus concluded that DEA is limited by soil P. The MBC was also significantly correlated with total P and DEA at $P < 0.01$ ($r = 0.70$; $r = 0.77$), suggesting that higher values for DEA in areas of P enrichment are due to increased microbial populations (Table 5-4).

Soils collected from along the transect, however, were not ideal for elucidating the effect of total P alone on microbial processes. Several other parameters changed as a result of nutrient loading, in concert with soil total P. McCormick and O'Dell (1996) found a significant trend in total dissolved nitrogen, calcium, iron, sodium, and soluble reactive P (SRP) of surface water along the transect. DeBusk and Reddy (1998) documented a significant difference in total N content of plant matter and total P content of living and senescent dead plant matter along the transect. Davis (1991) documented

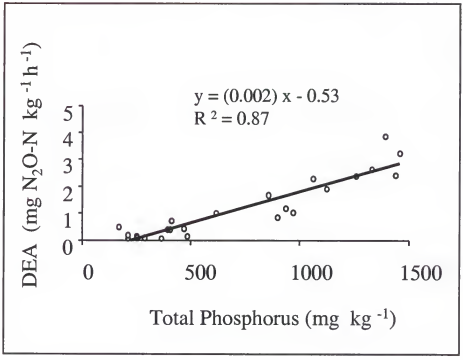

Figure 5-5. Plot of DEA vs total P for detritus, 0-10 and 10-30 cm soil interval from along the study transect in WCA-2A.

the change in plant community structure which had occurred over the past 30 years, manifesting itself as 3 distinct zones (dense cattail, mixed cattail and sawgrass, and sawgrass/periphyton communities). Craft and Richardson (1997) documented a gradient in Na and Ca content of the underlying peat. As a result of all these biological and physiochemical differences along the transect, a different approach was needed to truly discern the effects of P loading on DEA values.

Surface soils (0-3 cm), in experimental mesocosms, had been loaded with variable rates of P by the SFWMD (McCormick and O'Dell, 1996). Distribution of DEA values in the mesocom study showed no significant correlation with total soil P (Figure 5-6), though, total P was significantly ($P = 0.01$) correlated with P-loading rate (Table 5-4). However, the microbial biomass C is also significantly higher in soils with higher total P contents ($r = 0.50$). This suggests a P limitation to the microbial pool in natural Everglades peat soils. The fact that MBC and DEA are not significantly correlated could be due to the existence of a wide variety of functional microbial groups present in the soil (Drake et al, 1996). Denitrifiers make up a small percentage of the total microbial biomass where very little NO_3^- or O_2 is available to sustain microbial respiration. Duncan and Groffman (1994) also found no significant correlation between MBC and DEA for a natural and treatment wetland.

These results lead to the conclusion that the strong correlation of DEA with total P along the transect was simply an "incidental relationship" rather than causal. Nitrate N appeared to be the limiting factor as both N and P are loaded to WCA-2A in the drainage water. Phosphorus did not appear to be limiting to the denitrifier populations in the soil at the background level of NO_3^- loading (supplied through nitrification). If NO_3^- were

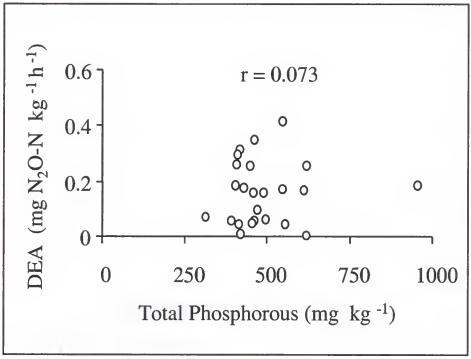

Figure 5-6. Plot of DEA vs total P for the surface (0-3 cm) soil from the P loading mesocosm experiment in WCA-2A. Samples were collected October 1996.

loaded at sufficiently high concentration so as not to be limiting, perhaps a P limitation might then become expressed.

Nutrient Addition Study

Potential denitrification rates, determined on surface soil samples spiked and incubated with various levels of NO_3-N, PO_4-P, and NO_3-N+PO_4-P, demonstrated significant differences over the range of N and N+P additions, but no significant differences for all levels of P. In addition, the two lowest levels of N and N+P additions also demonstrated no significant difference in denitrification rate over the control (no addition) or P only additions. Significant differences were only noted at the two highest levels of N (Table 5-6). This suggests that denitrification in Everglades soils is controlled by NO_3^- input, not P. Interestingly, there was also significantly higher denitrification for soils spiked with the two highest levels of N+P, compared to samples spiked solely with the same levels of N only. This suggests that as N becomes non-limiting, P then can become the limiting nutrient for denitrification. There were no significant differences between samples which had undergone N+P and N additions at the two lowest levels, suggesting NO_3^- limitation to denitrification.

A similar result was shown for DEA values on these soils (Table 5-6). The DEA was not significantly different for the controls, all P addition levels, and the lowest two levels of both N and N+P additions. The effect of N+P loading also demonstrated significantly higher rates of N_2O evolution than for N alone, but only at the highest level of additions.

Table 5-6. Potential denitrification rates and DEA for soil samples from the nutrient addition study. Letters following rates depict significant differences (same letter = not significant).

Porewater Concentration (mg L^{-1}) mg L^{-1}	Denitrification		DEA	
	----------mg N_2O-N kg^{-1} h^{-1}----------			
Control	n.d.	a	0.07	a
NO_3-N 1.0	0.03	a	0.02	a
NO_3-N 10.0	0.16	a	0.13	a
NO_3-N 50.0	16.7	b	3.66	b
NO_3-N 100	25.6	c	11.6	c
PO_4-P 0.1	n.d.	a	0.06	a
PO_4-P 1.0	n.d.	a	0.07	a
PO_4-P 5.0	n.d.	a	0.08	a
PO_4-P 10.0	n.d.	a	0.11	a
NO_3-N 1.0 + PO_4-P 0.1	0.01	a	0.07	a
NO_3-N 10.0 + PO_4-P 1.0	0.003	a	0.09	a
NO_3-N 50.0 + PO_4-P 5.0	26.3	c	4.38	b
NO_3-N 100 + PO_4-P 10.0	33.7	d	15.5	d

n.d. = not detectable

There were no significant differences between DEA values for N and N+P additions at the second highest level; however, both were significantly higher than for all low levels. These results demonstrate that DEA values of soils exposed to high levels of NO_3^- can become P limited (Table 5-6). The WCA-2A undergoes continual NO_3^- loading at low surface water concentrations (~ 1 mg N L^{-1}), however, and appeared to be primarily N limited.

Conclusions

The results of this study in suggest that the present NO_3^- loading rates to WCA-2A wetland have had an effect on the spatial and temporal distribution of soil DEA. The denitrifying microbial populations established in this wetland have responded to the last 40 years of NO_3^- loading, by maintaining highly active populations within a few km of the inflow point. All data suggest the exogenous NO_3^- is intercepted by this microbial pool and lost from the wetland as gaseous N_2 and N_2O. The majority of this NO_3^- is reduced in the detrital and surface (0-10 cm) soil layer. The distribution of DEA in surface soil decreased along the transect as a first-order decay function. During high loading seasons (summer), DEA distributions fit this exponential equation; however, during periods of low NO_3^- loading (winter) DEA distribution did not fit either a zero- or first-order model. This suggests that soil denitrifying enzymes are produced in response to increased NO_3^- loading.

Further investigations suggested that increased hydraulic loading into WCA-2A, thereby increasing NO_3^- loading, would stimulate a concomitant increase in the activity of denitrifying populations in soils and detritus. This increase in enzymes

activity/production would aid in the removal of additional NO_3^- associated with increased loading. Phosphorus loading appeared to have minimal effect on the level of soil DEA in the mesocosm study which suggests that the strong correlation between DEA and total P found in the transect study is coincident and not causal. The results of laboratory incubations with P and NO_3^- additions support this conclusion as different levels of P elicited no response in DEA. A P limitation on denitrification and DEA was demonstrated at high level of NO_3^- additions. These high rates of NO_3^- additions are significantly higher than N concentrations of impacted or unimpacted soils in the field, suggesting that *in situ* rates of NO_3^- reduction are NO_3^- limited in WCA-2a of Everglades soils and detritus.

This study suggests WCA-2A could potentially receive a far greater NO_3^- load without reaching a saturation limit on the potential denitrification capacity of soils within this 44,684 ha wetland. It has been demonstrated that the denitrifying enzyme assay can be used to discern areas of likely impact due to NO_3^- loading in flooded soils. This application of DEA might be utilized in other aquatic systems (eg. lakes and streams) to identify soils/sediments which are intercepting plumes of NO_3^- carried by surface water flow or by groundwater; however a calibration to determine baseline level DEA characteristics may be needed for each system.

CHAPTER 6
NITROGEN BUDGET IN SOILS ALONG A EUTROPHIC GRADIENT IN THE EVERGLADES WATER CONSERVATION AREA 2A

Introduction

The wide range of oxidation states of N in natural systems results in occurrence of a variety of N forms, whose transformations and their rates are, in turn, regulated by soil biological, chemical, and physical properties. The relative rates of the various transformation processes determine the extent to which N is conserved or lost from the system. Few studies on N cycling have developed N budgets by simultaneously measuring storages and transformation rates. In my study, I have attempted to quantify the storage and selected N transformations in detritus and soil components of the Everglades wetland. The N budget is confined to three specific zones in Water Conservation Area 2A of the Northern Everglades, representing the impacted, transitional, and unimpacted regions. The descriptive N budget model presented herein includes no mass balance, but simply demonstrates the potential N removal rates and attempts to identify the rate limiting process of N loss from the system.

Materials and Methods

Storages and N cycling processes were measured at eight locations in WCA-2A were used to construct the N budgets (Figure 6-1). Stations located 1.4 - 3.3 km from inflow represent the impacted zone characterized by thick, monotypic stands of *Typha sp.* and average surface soil (0-10 cm) total P concentrations of 1580 mg kg^{-1}. The mixed zone model represents stations located from 3.3 – 7.0 km from the inflow characterized by a vegetative community comprised of a mixture of cattail (*Typha, sp.*) and sawgrass (*Cladium, sp.*) and intermediate surface soil (0-10 cm) total P concentrations averaging 1220 mg kg^{-1}. The unimpacted zone is represented by stations located from 7.0- 10.1 km from inflow characterized by the historic, natural Everglades vegetative community consisting of small, stunted stands of sawgrass (*Cladium, sp.*) separate by shallow, open sloughs dominated by periphyton algae. Total P concentrations averaged 600 mg kg^{-1} in the unimpacted area.

The soil profile was subdivided into three major compartments; the detrital layer, 0-10 cm depth, and 10-30 cm depth. Previous work has suggested the size of the microbial biomass, microbial activity, and nutrient content/availability decreased with depth (DeBusk, 1996; DeBusk and Reddy, 1998). The influence of macrophyte roots on soil properties was also considered. Previous work by Miao and Sklar (1998) determined that 90 – 100 % of live roots were located in the top 20 cm of soil along the transect. Therefore, the top 30 cm of peat soil was considered to be the practical limit of direct rhizosphere influence. Additionally, the age of the peat soil increases with depth at each sampling station with decreasing total P concentration seen at depth. Therefore, the

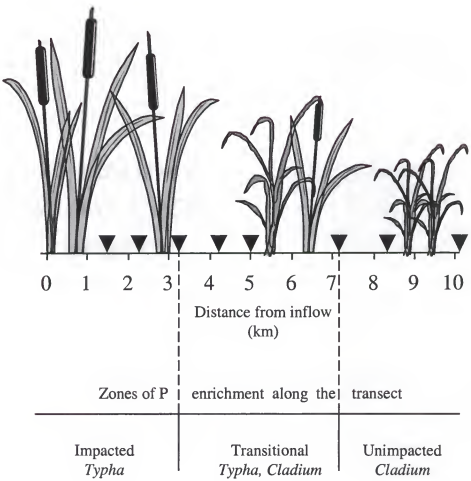

Figure 6-1. Distance along transect in WCA-2A depicting location of sampling stations (indicated by inverted triangles), extent of the impacted, transitional and unimpacted regions with the associated dominant macropyte community noted.

experimental design allowed for the separation of the soil volume into the aforementioned three soil compartments.

The following assumptions were made regarding the storage of N along the transect. All storages have been averaged over the year including RON (recalcitrant or residual organic N), MBN (microbial biomass N), SON (soluble organic N), and NH_4-N, all in units of g m^{-2} for each soil depth (Table 6-1). The RON includes remaining organic N compounds after MBN, SON, and NH_4^+ were subtracted out. Nitrate-N pools in each layer were considered to be negligible. This assumption is based upon low or undetectable values of NO_3^- in the pore water, on the exchange complex of the soil, and due to the significantly higher denitrification rates in comparison to nitrification rates. Bulk density of detritus was not measured along the transect therefore an estimation was made using water content/bulk density relationships from the soil resulting in an approximation of 0.04 g cm^{-3} for all stations. The detrital layer varied in mean thickness along the transect; 10 cm in the impacted zone, 4 cm in the transitional zone and 1 cm in the unimpacted zone. This observation is consistent with earlier findings of higher soil accretion rates at stations proximal to the inflow and lowest rates located distally (Reddy et al., 1993).

Values for the standing live and standing dead macrophytes compartment are in units of g N kg^{-1} dry weight of plant material (DeBusk, 1996). The difference in total N content of the live and dead standing material is due to both leaching and translocation processes. Literature values for standing crop biomass are a bit misleading in this case and therefore are not included, as they do not clearly delineate the effect of the macrophyte community structure on nutrient cycling. Taken on a standing crop basis, the

Table 6-1. Mean soil storage values from along the transect in WCA-2A followed by one standard deviation in parentheses (RON = recalcitrant organic nitrogen; MBN = microbial biomass nitrogen; SON = soluble organic nitrogen).

Soil Depth	Distance	Bulk Density	total N	RON	MBN	SON	NH_4^+-N
	km	g cm^{-3}	g N m^{-2}				
Detritus							
10 cm	1.4-3.3	0.040	98.3	85.8	5.70	4.98	1.83
			(12.2)	(7.57)	(2.07)	(1.65)	(0.91)
4 cm	3.3-7.1	0.040	34.2	30.4	1.79	1.34	0.63
			(4.86)	(3.27)	(0.83)	(0.49)	(0.27)
1 cm	7.1-10.1	0.040	9.27	8.47	0.34	0.33	0.13
			(1.54)	(1.15)	(0.19)	(0.14)	(0.06)
0-10 cm	1.4-3.3	0.050	142	135	2.34	4.26	0.64
			(18.8)	(16.6)	(1.00)	(0.68)	(0.48)
0-10 cm	3.3-7.1	0.066	190	183	1.93	3.93	0.83
			(19.3)	(17.0)	(1.09)	(0.85)	(0.37)
0-10 cm	7.1-10.1	0.063	185	177	2.11	4.27	0.79
			(16.5)	(18.6)	(1.17)	(0.89)	(20.27)
10-30 cm	1.4-3.3	0.095	605	591	2.15	10.9	1.18
			(65.1)	(63.5)	(0.64)	(0.48)	(0.53)
10-30 cm	3.3-7.1	0.088	532	520	1.90	9.62	0.98
			(86.2)	(84.5)	(0.75)	(0.55)	(0.44)
10-30 cm	7.1-10.1	0.081	455	443	1.72	9.42	0.77
			(65.7)	(63.4)	(1.03)	(0.94)	(0.30)

unimpacted region has equal total plant (including roots, rhizomes, leaves and stalks) biomass as the impacted region in vegetated areas (Miao and Sklar, 1998). However, there are some important differences between the two areas which are not accounted for in this estimate. First, the estimates were produced from vegetative areas in both areas, however, in reality the percentage of open, non-vegetated slough areas is far greater in the unimpacted region. Additionally, *Typha* have been shown to grow faster, have shorter life spans, and greater nutrient content (N and P) in leaves than *Cladium* along the transect (Miao and Sklar, 1998). Therefore, the yearly vegetative yield of *Typha* is higher than *Cladium* leading to higher detrital production of higher nutrient content in the impacted region. This higher productivity leads to increased detrital material.

All N cycling processes/rates are in unit of $g\ m^{-2}\ d^{-1}$ in all layers (Table 6-2). Organic N mineralization rates under aerobic and NO_3^- reducing conditions were considered only in the detrital layer in each zone. Mineralization under SO_4^{2-} reducing and methanogenic conditions only, were considered the predominant mechanisms for NH_4^+ production in the 0-10 cm and 10-30 cm soil layers. This assumption was based on the high soil oxygen demand (microbial respiration) and denitrification enzyme activity of the detritus which would likely consume any O_2 or NO_3^- respectively, diffusing downward into the soil from the water column (Chapters 3 and 4).

Experimentally, two sets of nitrification and denitrification rates were determined. In the case of nitrification, the rate of NO_3^- production over the first 1-2 days of incubation under well stirred, aerobic conditions in the reactors (Chapter 4) were included. These rates are clearly an overestimation of *in situ* nitrification rates, which are

Table 6-2 . Mean rates of nitrogen cycling processes measured along the transect in WCA-2A with one standard deviation in parentheses (NIT = nitrification rate; DEA = denitrification enzyme assay).

Soil		----- Mineralization condition -----				Initial	
Depth	Distance	O_2	NO_3^-	SO_4^{2-}	CO_2	NIT	DEA
	km	---------- g N m^{-2} d^{-1} ----------					
Detritus							
10 cm	1.4-3.3	0.971	0.268	0.139	0.088	0.134	0.311
		(0.06)	(0.01)	(0.01)	(0.02)	(0.06)	(0.056)
4 cm	3.3-7.1	0.411	0.099	0.063	0.027	0.034	0.096
		(0.70)	(0.17)	(0.01)	(0.03)	(0.01)	(0.044)
1 cm	7.1-10.1	0.080	0.018	0.013	0.008	0.012	0.013
		(0.11)	(0.19)	(0.10)	(0.02)	(0.01)	(0.008)
0-10 cm	1.4-3.3	0.744	0.075	0.035	0.033	0.037	0.161
		(0.03)	(0.15)	(0.04)	(0.01)	(0.01)	(0.123)
0-10 cm	3.3-7.1	0.901	0.161	0.084	0.012	0.089	0.170
		(0.05)	(0.06)	(0.05)	(0.01)	(0.10)	(0.110)
0-10 cm	7.1-10.1	0.912	0.058	0.038	0.034	0.086	0.084
		(0.02)	(0.02)	(0.01)	(0.003)	(0.07)	(0.052)
10-30 cm	1.4-3.3	1.230	0.074	0.039	0.027	0.037	0.170
		(0.21)	(0.007)	(0.008)	(0.02)	(0.004)	(0.209)
10-30 cm	3.3-7.1	1.425	0.084	0.082	0.017	0.040	0.147
		(0.35)	(0.08)	(0.10)	(0.006)	(0.014)	(0.175)
10-30 cm	7.1-10.1	1.156	0.085	0.077	0.020	0.030	0.141
		(0.02)	(0.09)	(0.12)	(0.001)	(0.007)	(0.148)

likely, controlled by the rate of O_2 transfer into the soil through the macrophyte roots. However, the potential nitrification rates were 11 times higher than these initial measured rates and likely do not represent *in situ* rates, even under completely drained soil conditions. The DEA rates were included as the best estimate for *in situ* denitrification rates as the denitrifying potential rates determined under non-limiting NO_3^- concentrations are unrealistic, and would not approximate even the most ideal field conditions.

Results and Discussion

The greatest effect of P enrichment on the biogeochemical cycling of N in the Everglades can be seen in the impacted zone dominated by *Typha* (Figure 6-2). The detrital layer is the most microbially active compartment in the cycling of N (Table 6-1). Due to the thickness of the detrital component in this region, the importance of both N storage and transformation processes in this layer become evident when the data are expressed on an areal basis. The MBN pool is twice the size of the pool in the 0-10 cm depth and almost 3 times greater than the total microbial biomass present in the 10-30 cm layer (which is double the thickness). The organic N mineralization rates are also higher in the detritus than in the underlying soil, evidenced by the higher mass of extractable NH_4^+ and results of experimental incubations (Chapter 2 and 3). The initial nitrification rates are one order of magnitude higher in the detritus when compared to the underlying soil depths. The higher nitrification rates in the detritus are responsible for NH_4^+ consumption. It is likely the reason that there is not a greater difference in the mass of

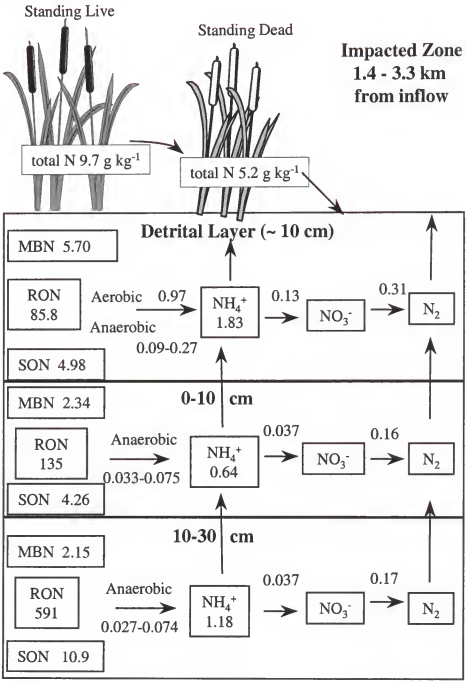

Figure 6-2. Box model of N cycling processes and storages at 1.4-3.3 km from the inflow in WCA-2A. Storages are in units of g m^{-2} and rates are in units of g m^{-2} d^{-1}. Total N of plant tissue is in units of g kg^{-1} dry.

extractable NH_4^+ between the detrital and soil compartments is due to the autotrophic consumption of NH_4^+ in the surface as the substrate for nitrification.

There is a noticeable decrease in MBN in the detritus as you move from the impacted region to the transition area and further to the unimpacted areas. The MBN compartment in the transitional region is ~ 4 times smaller (Figure 6-3) and 18 times smaller (Figure 6-4) in the unimpacted region compared with the impacted zone. Nitrification rates are also an order of magnitude greater in the impacted region compared to the transitional and unimpacted region. As established in chapter 5, the denitrification enzyme activity is highest in the impacted region, due primarily to the stimulation of the functional microbial consortia to NO_3^- inputs in the inflow water. The DEA activity of the large detrital component in the impacted region essentially prevents exogenous NO_3^- loading to the rest of the wetland. This conclusion is supported by nitrification and denitrification rates of similar magnitude, which suggests the denitrifying enzyme concentration is stimulated by NO_3^-, produced *in situ* by nitrification in the unimpacted region.

The microbial pool is the single most important component driving the N cycle. The microbial biomass has been described as the primary agent of the soil ecosystem responsible for litter decomposition, nutrient cycling, and energy flow (Wardle, 1992). In demonstrating the effects of P loading on N cycling, the microbial pool has been set as the central force which drives the nutrient cycling and ecosystem changes due to P loading in the Everglades (Figure 6-5). The activity and size of the microbial pool have increased under increased additions of P (Chapter 2). This increased activity has led to an increase in the release of inorganic N from the organic pool (Chapter 2).

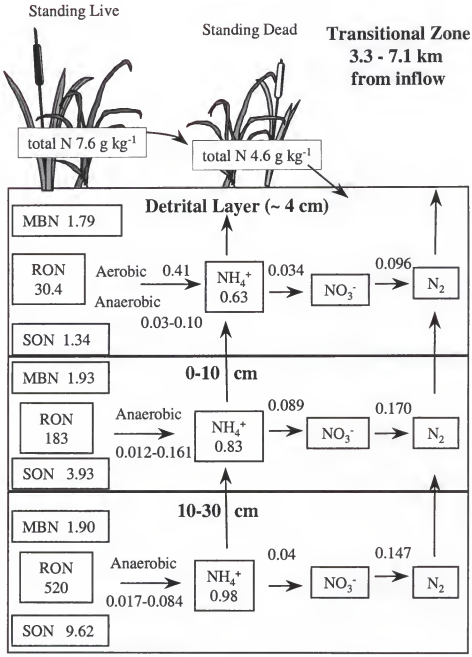

Figure 6-3. Box model of N cycling processes and storages at 3.3-7.1 km from the inflow in WCA-2A. Storages are in units of g m^{-2} and rates are in units of g m^{-2} d^{-1}. Total N of plant tissue is in units of g kg^{-1} dry.

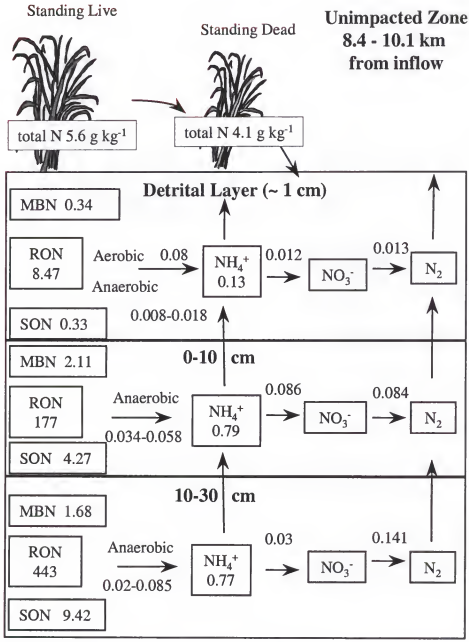

Figure 6-4. Box model of N cycling processes and storages at 7.1-10.1 km from the inflow in WCA-2A. Storages are in units of g m^{-2} and rates are in units of g m^{-2} d^{-1}. Total N of plant tissue is in units of g kg^{-1} dry.

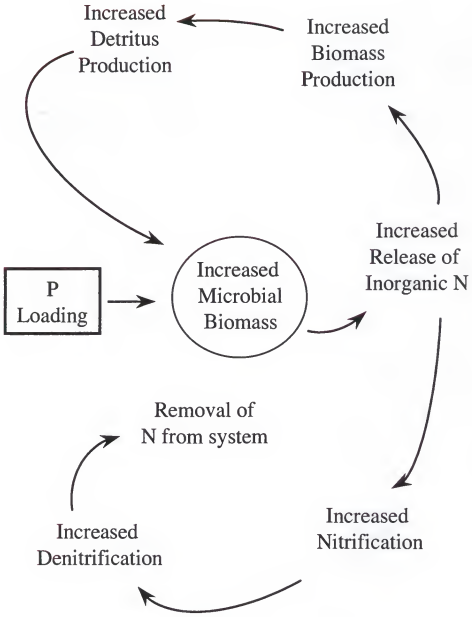

Figure 6-5. Effects of phosphorus loading on cycling of nitrogen from along the transect in WCA-2A.

The larger, microbial pool also has led to increased rates of potential nitrification (Chapter 4), leading to removal of N from the system by denitrification. Nitrification rates, however, are lower than potential organic N mineralization rates due to the anaerobic status of the soil, leading to the build up of NH_4^+ in the soil, where it can be utilized by macrophytes. Nitrification is the rate limiting process controlling N loss from the system. This fact is evident in the non-existent NO_3^--N pool that apparently is quickly denitrified as soon as it is produced in these waterlogged soils. The effect of drainage on the total N turnover can be estimated by imputing the maximum nitrification values determined experimentally (Chapter 4), which were one order of magnitude greater than the initial nitrification rates.

The most useful measure for determining the contribution of the macrophytes to the detritus would be annual biomass yield of which there are none that I have located in the literature. The differences in biomass yield between the *Typha* and *Cladium* vegetative communities are due to differences in physiological/life history between the plant types. Cattails grow fast, have high population growth rates, short life cycles, high reproductive output and rapid response to resource availability. Conversely, sawgrass have been found to grow slowly, have low population growth, long life cycles, low reproductive yield, and respond slowly to resource availability (Miao and Sklar, 1998). Consequently, future needs for research need to focus on defining the feedback of macrophytes to the overall nutrient cycling of wetlands.

Conclusions

The rates for N cycling processes, presented in the preceding chapters, were determined in most cases with non-limiting substrates, homogenous soil conditions, and under well-stirred/shaken incubations in order to negate diffusion limitations. Therefore, the budget model should be viewed with some caution, as absolute organic N turnover rates/removal rates are likely an overestimation of the *in situ* rates. The greatest contribution of the model lies in the determination of relative changes in processes as well as absolute storage of N in response to the nutrient gradient in WCA-2A.

Additionally, this approach was useful in delineating the overall rate limiting process of N loss from the wetland ecosystem. Nitrification rates of soil and detritus clearly control the removal rate of NH_4^+. This qualitative model can be used to predict order of magnitude changes in N turnover prompted by changes in management practices which might result in changes in water column thickness/duration or inorganic electron acceptor concentrations, for example.

CHAPTER 7
SUMMARY AND CONCLUSIONS

The results of laboratory and field studies were conducted to examine the influence of phosphorus enrichment on nitrogen cycling in Everglades wetland soils. The specific objectives were outlined in Chapter 1 and addressed by experimental studies contained in Chapters 2-5. A summary of results as they relate to the study objectives is presented.

Objectives

1) Determine the influence of P loading on potential ammonification (N mineralization) of soil and detritus under aerobic and anaerobic conditions.

Phosphorus enrichment of soil and detritus was found to have an indirect controlling effect on organic N mineralization rates of native soil organic matter. Total P caused increased release rates of inorganic N in the form of NH_4^+ for the anaerobic condition and NO_3^- for the aerobic condition through a stimulation on the size and/or activity of the microbial pool in the P-limited soil. The detrital was found to be the most microbiologically active soil component releasing the greatest amount of inorganic N. The 0-10 cm soil depth contributed less to the overall inorganic N budget with the least amount of inorganic N released from the 10-30 cm soil depth. The organic N

mineralization rates decreased with increasing distance from the inflow, mimicking the total P distribution in the soil and detritus.

Under anaerobic conditions, the dominance of inorganic electron acceptors affected the rate of NH_4^+ release from the organic pool. Organic N mineralization under NO_3^- and SO_4^{2-} conditions were higher than release rates under methanogenic conditions. Consequently, loading of NO_3^- and SO_4^{2-} to the Everglades wetlands could still lead to increased N turnover rates even when water levels are held high to prevent the aerobic oxidation of the organic soil.

2) Determine the influence of P loading on potential nitrification rates of soil and detritus.

Nitrification was found to be limited by aeration status in these saturated high organic matter soils. Initial rates of nitrification were therefore a function of the size and activity of the autotrophic microbial pool present at each soil depth. Potential rates of nitrification were ~ 11 times greater than the initial rates. The initial nitrification rates were correlated to the nitrifying substrate (NH_4^+) concentration in the soil which was a function of the heterotrophic microbial component. Therefore, nitrification rates were indirectly affected by the stimulatory effect of P on the heterotrophic microbial pool providing the substrate for nitrification.

3) Determine the influence of P loading on denitrifying potential and activity of the denitrifying enzyme of soil and detritus.

The total P content appeared to have a stimulatory effect on the denitrifying potential of soils and detritus. Rates decreased exponentially with increasing distance from the inflow point. Phosphorus enrichment was well correlated to DEA along the transect, however this was a coincidental, not causal relationship. Results of laboratory incubations demonstrated that DEA was indeed a function of NO_3^- loading, not P loading as initially proposed.

4) Construct a nitrogen budget for soils and identify the limiting process regulating overall N loss from the system.

The nitrogen budget constructed from along the transect demonstrated that P enrichment has influenced the size of the storage of N within the wetland system. The impact of nutrient loading on *Typha*, has resulted in a thick, microbially active detrital layer within the impacted region. Consequently, organic N turnover has increased creating a feedback loop providing inorganic N in the form of NH_4^+ for fertilization of the macrophytes present.

The limiting N cycling process for the Everglades wetland soil is nitrification. Results have shown that any increase in NO_3^- levels will stimulate a concomitant increase in denitrifier activity in the soil. Potential nitrification rates are one order of magnitude higher than initial rates. Therefore, any water management strategy which results in a lowering of the water table and exposing the wetland soil surface, will lead to increased loss of N from the system through the nitrification-denitrification coupled reactions.

Conclusions

Phosphorus enrichment of the wetland soil appears to have influenced not only the structure and composition of the vegetative community in WCA-2A, but also the biogeochemical cycling of C (DeBusk, 1996) and N within the system. The current restoration strategy is primarily aimed at reducing P levels in the agricultural drainage waters just prior to entering the Water Conservation Areas through the construction of several front-end treatment wetlands. Research presented herein suggests that some attention and concern should be afforded the issue of P-related nutrient turnover, in particular, release of inorganic N.

Recent research by Fisher (1997) suggests that P will continually be released from soils within the impacted region of WCA-2A for a significant period of time. As this mobile P fraction is carried further south into low soil P areas, soil microbial activity will increase resulting in the alteration of biotic communities including the invasion of *Typha* into areas currently dominated by *Cladium*. The *Typha* will be "fertilized" through the feedback mechanism of increasing microbial-mediated release of inorganic N from the soil and detritus. Therefore, if P-loading continues further into the Everglades, it is likely that ecosystem changes will continue due, in part, to changes in N cycling.

REFERENCES

Addiscott, T. M. 1983. Kinetics and temperature relationships of mineralization and nitrification in Rothamsted soils with differing histories. J. Soil Sci. 34:343-353.

Alef, K., T. Beck, L. Zelles and D. Kleiner. 1988. A comparison of methods to estimate microbial biomass and N-mineralization in agricultural and grassland soils. Soil Biol. Biochem. 20:561-565.

Alef, K. and D. Kleiner. 1986. Arginine ammonification, a simple method to estimate microbial activity potentials in soils. Soil Biol. Biochem. 18:233-235.

Amador, J. A. and R. D. Jones. 1993. Nutrient limitations on microbial respiration in peat soils with different total phosphorus content. Soil Biol. Biochem. 25:793-801.

Amador, J. A. and R. D. Jones. 1997. Response of carbon mineralization to combined changes in soil moisture and carbon-phosphorus ratio in a low phosphorus histosol. Soil Sci. 162:275-282.

American Public Health Association. 1992. Standard methods for the examination of water and wastewater. 18th ed. Am. Public Health Asso., American Waterworks Asso., and Water Environment Fed. (Eds). APHA, Washington, D.C.

Anderson, T.H. and K. H. Domsch. 1985. Determination of the ecophysiological maintenance carbon requirements of soil microorganisms in a dormant state. Biol. Fert. Soil. 1:81-89.

Anderson, T.H. and K. H. Domsch. 1989. Ratio of microbial biomass carbon to total organic carbon in arable soils. Soil Biol. Biochem. 21:471-479

Balderston, W. L., B. Sherr, and W. J. Payne. 1976. Blockage by acetylene of nitrous oxide reduction in *Psuedomonal perfectomarinus*. Appl. Environ. Micro. 31:504-504.

Bell, R. T. and I. Ahlgren. 1987. Thymidine incorporation and microbial respiration in the surface sediment of a hypereutrophic lake. Limnol. Oceanogr. 32:476-482.

Biederbeck, V. O., C. A. Campbell, and R. P. Zentner. 1984. Effects of crop rotation and fertilization on some biological properties of a loam in southwestern Saskatchewan. Can. J. Soil Sci. 64:355-367.

Brady N. C. and R. R. Weil, 1996. The nature and properties of soils 11th edition Prentice-Hall, Inc. NJ. 740 pp.

Bremner, J. M. and C. S. Mulvaney. 1982. Nitrogen-total. *In* A.L. Page et al. (ed.) Methods of soil analysis. Part 2. 2nd ed. Agronomy 9:595-624.

Brock, T. D., M. T. Madigan, J. M. Martinko and J. Parker. 1994. Biology of microorganisms. 7th ed. Prentice Hall, Englewood Cliffs, NJ.

Brookes, P. C., D. S. Powlson, D. S. Jenkinson. 1984. Phosphorous in the soil microbial biomass. Soil Biol. Biochem. 16:169-175.

Brookes, P.C., A. Landman, G. Pruden, and D. S. Jenkinson. 1985. Chloroform fumigation and the release of soil nitrogen: a rapid direct extraction method to measure microbial biomass nitrogen in soils. Soil Biol. Biochem. 17:83-842.

Burton, D. L. and E. G. Beauchamp. 1985. Denitrification rate relationships with soil parameters. Comm. Soil Sci. Plant Anal. 16:539-549.

Christiansen, S., S. Simkins, and J. M. Tiedje. 1990a. Temporal patterns of soil denitrification:their stability and causes. Soil. Sci. Soc. Am. J. 54:1614-1618.

Christiansen, S., S. Simkins, and J. M. Tiedje. 1990b. Spatial variation of denitrification: Dependency on activity centers on the soil environment. Soil. Sci. Soc. Am. J. 54:1608-1613.

Cooper, A. B. 1990. Nitrate depletion in the riparian zone and stream channel of a small headwater catchment. Hydrobiologia 202:13-26.

Craft, C.B. and C. J. Richardson. 1993. Peat accretion and N, P and organic C accumulation in nutrient-enriched Everglades peatlands. Ecol. Appl. 3:3:446-458.

Craft, C.B. and C. J. Richardson. 1997. Relationships between soil nutrients and plant species composition in Everglades peatlands. J. Environ. Qual. 26:224-232.

Damman, A. W. H. 1988. Regulation of nitrogen removal and retention in Sphagnum bogs and other peatlands. OIKOS 51:291-305.

D'Angelo, E. M. and K. R. Reddy. 1994. Diagenesis of organic matter in a wetland receiving hypereutrophic lake water: II. Role of inorganic electron acceptors. J. Environ. Qual 23:937-943.

D'Angelo, E. M. and K. R. Reddy. 1999. Regulators of heterotrophic microbial potentials in wetland soils. Soil Biol Biochem. (in press)

Davis, J. H., Jr. 1943. The natural features of southern Florida especially the vegetation and the Everglades. Florida Geological Survey: bulletin 25, 311 pp.

Davis, S. M. 1991. Growth, decomposition and nutrient retention of *Cladium jamaicense* Cranz. and *Typha domingensis* Pers. in the Florida Everglades. Aquat. Bot. 40:203-224.

DeBusk, W. F. 1996. Organic matter turnover along a nutrient gradient in the Everglades. Ph.D. Dissertation. University of Florida, Gainesville, Florida, USA. 176 pp.

DeBusk, W. F., K. R. Reddy, M. S. Koch, and Y. Wang. 1994. Spatial distribution of soil nutrients in a northern Everglades Marsh: Water Conservation Area 2A. Soil Sci. Soc. Am. J. 58:543-552.

DeBusk, W. F. and K. R. Reddy. 1998. Turnover of detrital organic carbon in a nutrient-impacted Everglades marsh. Soil Sci. Soc. Am. J. 62:1460-1468.

Drake, H. L., N. G. Aumen, C. Kuhner, C. Wagner, A. Grießhammer, and M. Schmittroth. 1996. Anaerobic microflora of Everglades sediment: effects of nutrients on population profiles and activities. Appl. Environ. Microbiol. 62:486-493.

Duncan, C. P. and P. M. Groffman. 1994. Comparing microbial parameters in natural and constructed wetlands. J. Environ. Qual. 23:298-305.

Espinoza, L. 1997. Fate of nitrogen and metals following waste applications to some Florida soils. Ph.D dissertation, University of Florida, Gainesville, FL. 152 p.

Fisher, M. M. 1997. Estimating landscape scale flux of phosphorus using geographic information systems (GIS). M.S. Thesis. University of Florida, Gainesville, Florida, USA. 148 pp.

Flora, M. D., D. R. Walker, D. R. Burgess. D. J. Schiedt, and R. G. Rice. 1986. The response of experimental channels in Everglades National Park to increased nitrogen and phosphorus loading, Water Resources Report No. 86-6. National Park Service, Water Resources Division, Colorado State University, Fort Collins.

Franzluebbers, A. J., R. L. Haney, F. M. Hons, and D. A. Zuberer. 1996. Determination of microbial biomass and nitrogen mineralization following rewetting of dried soil. Soil Sci. Soc. Am. J. 60:1133-1139.

Franzluebbers, A. J., F. M. Hons, and D. A. Zuberer. 1995. Soil organic carbon, microbial biomass, mineralizable carbon and nitrogen in sorghum. Soil Sci. Soc. Am. J. 59:460-466.

Fuhrman, J. 1987. Close coupling between release and uptake of dissolved free amino acids in seawater studied by an isotope dilution approach. Mar. Ecol. Prog. Ser. 37:45-52.

Fuhrman, J. and T. M Bell. 1985. Biological considerations in the measurement of dissolved free amino acids in seawater and implications for chemical and microbiological studies. Mar. Ecol. Prog. Ser. 25:13-21.

Gale, P.M., I. Devai, K. R. Reddy, and D. A. Graetz. 1993. Denitrification potential of soils from constructed and natural wetlands. Ecol. Engin. 2:119-130.

Gale, P. M. and J. T. Gilmour. 1988. Net mineralization of carbon and nitrogen under aerobic and anaerobic conditions. Soil Sci. Soc. Am. J. 52:1006-1010.

Gale, P. M., K. R. Reddy, and D. A. Graetz. 1992. Mineralization of sediment organic matter under anoxic conditions. J. Environ. Qual. 21:394-400.

Gardner, W. S., J. F. Chandler, G. A. Laird, and D. Scavia. 1986. Microbial response to amino acid additions in Lake Michigan:grazer control and substrate limitation of bacterial populations. J. Great Lakes Res. 12(3):161-174.

Gardner, W. S., J. F. Chandler, G. A. Laird, and D. Scavia. 1987. Sources and fate of dissolved free amino acids in epilimnetic Lake Michigan water. Limnol. Oceanogr. 32(6):1353-1362.

Gardner, W. S., J. F. Chandler, G. A. Laird. 1989. Organic nitrogen mineralization and substrate limitation of bacteria in Lake Michigan. Limnol. Oceanogr. 34:478-485.

Groffman, P. M. 1987. Nitrification and denitrification in soil: a comparison of enzyme assay, incubation and enumeration methods. Plant and Soil 97:445-450.

Groot, J. J. and V. J. G. Houba. 1995. A comparison of different indicies for nitrogen mineralization. Biol. Fertil. Soil 19:1-9.

Hollibaugh 1978. Nitrogen regeneration during the degradation of several amino acids by plankton communities collected near Halifax, Nova Scotia, Canada. Mar. Biol. 45:191-201.

Hopkins, D. W., R. W. Dowd, and R. S. Shiel. 1997. Comparison of D- and L-amino acid metabolism in soils with differing microbial biomass and activity. Soil Biol. Biochem.29:23-29.

Hopkins, D. W. and K. E. Ferguson. 1994. Substrate induced respiration in soil amended with different amino acid isomers. Appl. Soil Ecol. 1:75-81.

Hopkins, D. W., B. L. Isabella, and S. E. Scott. 1994. Relationship between microbial biomass and substrate induced respiration in soils amended with D- and L-isomers of amino acids. Soil Biol. Biochem. 26:1623-1627.

Hossain, A. K. M. A., R. J. Raison, and P. K. Khanna. 1995. Effects of fertilizer application and fire regime on soil microbial biomass carbon and nitrogen and nitrogen mineralization in an Australian subalpine eucalypt forest. Biol. Fertil. Soil 19:246-252.

Hue, N. V. and F. Adams. 1984. Effect of phosphorus level on nitrification rates in three low-phosphorus ultisols. Soil Sci. 137:324-331.

Humphrey, W. D. and D. J. Pluth. 1996. Net nitrogen mineralization in natural and drained fen peatlands in Alberta, Canada. Soil Sci. Soc. Am. J. 60:932-940.

Inubushi, K. F. Shibahara, T. Tsuji, and M. Umebayashi. 1992. Assay for microbial nitrogen in unflooded paddy soil by chloroform fumigation-extraction method. Jpn. J. Soil Sci. Plant Nutr. 63:342-344.

Isirimah, L. and D. R. Keeney. 1979. Nitrogen transformations in aerobic and waterlogged histosols. Soil Sci. 115:123-129.

Jenkinson, D. S. 1988. Determination of microbial biomass carbon and nitrogen in soil. In: Wilson J. R. (ed) Advances in nitrogen cycling in agricultural ecosystems. CAB International, Wallingford 368-386.

Joergensen, P. G., P. C. Brookes, and D. S. Jenkinson. 1990. Survival of the soil microbial biomass at elevated temperatures. Soil Biol. Biochem. 22:1023-1027.

Keeney, D. R. 1982. Nitrogen-Available Indices *In* Methods of Soil Analysis, Part 2. Chemical and Microbial Properties. Agronomy No. 9 (A.L. Page, R.H. Miller and D.R. Keeney Eds.) 2nd Ed. pp 711-734. American Society of Agronomy, Madison.

Koch, M. S. and K. R. Reddy. 1992. Distribution of soil and plant nutrients along a trophic gradient in the Florida Everglades. Soil. Sci. Soc. Am. J. 56:1492-1499.

Kuo, S. 1996. Phosphorus . p. 869-919. *In* J. M Bigham (ed.) Methods of Soil Analysis . Part 3. Soil Sci. Soc. Amer., Inc. Madison, WI.

Martin, K., L. L. Parsons, R. E. Murray, and M. S. Smith. 1988. Dynamics of soil denitrifier populations: relationships between enzyme activity, most-probable-number counts and actual N Loss. Appl. Environ. Micro. 54:2711-2716.

McCormick, P. and O'Dell, M. 1996. Quantifying periphyton responses to phosphorus in the Florida Everglades: a synoptic-experimental approach. J. N. Am. Benthol Soc. 15:450-468.

McLatchey, G. P. and K. R. Reddy. 1998. Regulation of organic matter decomposition and nutrient release in a wetland soil. J. Environ. Qual. 27:1268-1274.

Miao, S and W. F. DeBusk. 1998. Effects of phosphorus enrichment on structure and function of sawgrass and cattail communities in Florida wetlands. *In* Phosphorus Biogeochemistry in Subtropical Ecosystems. Florida as a Case Example. K. R. Reddy, G. A. O'Connor and C. L. Schelske (eds), CRC/Lewis Publ. 1998 (in press).

Miao, S and F. H Sklar. 1998. Biomass and nutrient allocation os sawgrass and cattail along a nutrient gradient in Florida Everglades. Wetland Ecosystem and Management (in press).

Munevar, F. and A. G. Wollum. 1977. Effects of the addition of phosphus and inorganic nitrogen carbon and nitrogen mineralization in adepts from Columbia. . Soil Sci. Soc. Am. J. 41:540-545.

Nair, M. K. 1996. Nitrogen cycling in Everglades peat soil. Ph.D. dissertation, Florida International University. Miami, FL. 92 p.

Nyhan, J. W. 1976. Influence of soil temperature and water tension on the decomposition rate of carbon-14 labeled herbage. Soil Sci. 121:288-293.

Parkin, T.B. 1987. Soil microsites as a source of denitrification variability. Soil Sci. Soc. Am. J. 51:1194-1199.

Parsons, L.L., R. E. Murray, and M. S. Smith. 1991. Soil denitrification dynamics: spatial and temporal variations of enzyme activity, populations and nitrogen gas loss. Soil Sci. Soc. Am. J. 55: 90-95.

Perucci, P. 1990. Effects on the addition of municipal solid waste compost on microbial biomass and enzyme activity in soil. Biol. Fert. of Soil 10:221-226.

Prescott, C. E., J. P. Corbin, and D. Parkinson. 1992. Immobilization and availability of N and P in the forest floors of fertilized Rocky Mountain coniferous forests. Plant and Soil 143:1-10.

Reddy, K. R. and D. A. Graetz. 1988. Carbon and nitrogen dynamics in wetland soils. p. 307-318. In D. D. Hook et al. (ed.) The Ecology and Management of Wetlands. Volume I:Ecology of Wetlands. Timber Press, Portland, Oregon.

Reddy, K. R. and W. H. Patrick. 1975. Effect of alternate aerobic and anaerobic conditions on redox potential, organic matter decomposition, and nitrogen loss in a flooded soil. Soil Biol. Biochem. 7:87-94.

Reddy, K. R., W. H. Patrick, and R. E. Phillips. 1980. Evaluation of selected processes controlling nitrogen loss in a flooded soil. Soil Sci. Soc. Am. J. 44:1241-1246.

Reddy, K. R. and W. H. Patrick. 1984. Nitrogen transformations and loss in flooded soil and sediments. CRC Critical Reviews in Environ. Control. 13:273-309.

Reddy, K. R., W. H. Patrick, and C. W. Lindau. 1989. Nitrification-denitrification at the plant root sediment interface in wetlands. Limnol. Oceanogr. 34:1004-1013.

Reddy, K. R., P. S. C. Rao, and R. E. Jessup. 1990. Transformations and transport of ammonium nitrogen in a flooded organic soil. Ecol. Modeling 51:205216.

Reddy, K. R., R. D. DeLaune, W. F. DeBusk, and M. S. Koch. 1993. Long term nutrient accumulation rates in the Everglades. Soil Sci. Soc. Am. J. 57:1147-1155.

Reddy, K. R., J. R. White, A. L. Wright and T. T. Chua. 1999. Influence of phosphorus loading on microbial processes in soil and water column of wetlands. *In* Phosphorus Biogeochemistry in Subtropical Ecosystems. Florida as a Case Example. K. R. Reddy, G. A. O"onnor and C. L. Schelske (eds), CRC/Lewis Publ. 1998 (in press).

Robertson, G. P. 1989. Nitrification and denitrification in humid tropical ecosystems: Potential controls on nitrogen retention p. 55-69. In Procter (ed.) Mineral nutrients in tropicalforest and savanna ecosystems. Blackwell Sci. Publ., Oxford.

Robertson, K., J. Schnurer, M. Clarholm, T. A. Bonde, and T. Rosswall. 1988. Microbial biomass in relation to C and N mineralization during laboratory incubations. Soil Bio. Biochem. 20:281-286.

Ross, D. J. and T. W. Speir, H. A. Kettles, and A. D. Mackay. 1995. Soil microbial biomass, C and N mineralization and enzyme activities in a hill pasture:influence of season and slow-release P and S fertilizer. Soil Biol. Biochem. 27:1431-1443.

Sarathchandra, S. U., K. W. Perrott, and R. A. Littler. 1989. Soil microbial biomass: influence of simulated temperature changes on size, activity and nutrient content. Soil Biol. Biochem. 21:987-993.

Schipper, L.A., A. B. Cooper, C. G. Harfoot, and W. J. Dyck. 1993. Regulators of denitrification in an organic riparian soil. Soil Biol. Biochem. 25:925-933.

Schnurer, J., M. Clarholm, and T. Rosswall. 1985. Microbial biomass and activity in an agricultural soil with different organic matter contents. Soil Biol. Biochem. 17:611-618.

Sexstone, A. J., T. B. Parkin, and J. M Tiedje. 1985. Temporal response of soil denitrification rates to rainfall and irrigation. Soil. Sci. Soc. Am. J. 49:99-103.

Smith, M.S. and L. L. Parsons, 1985. Persistence of denitrifying enzyme activity in dried soils. Appl. Environ. Micro. 49:316-320.

Smith, M.S. and J. M. Tiedje. 1979. Phases of denitrification following oxygen depletion in soil. Soil Biol. Biochem. 11:261-267.

Sorensen, J., B. B. Jorgensen, and N. P. Revsbech. 1979. A comparison of oxygen, nitrate and surfate respiration in a coastal marine sediment. Microbial Ecol. 5:105-115.

Sparling, G. P. and A. W. West. 1989. Importance of soil water content when estimating soil microbial C, N, and P by the fumigation-extraction method. Soil Biol. Biochem. 21:245-253.

Sparling, G. P., K. N. Whale, and A. J. Ramsay. 1985. Quantifying the contribution from the soil microbial biomass to the extractable P levels of fresh and air-dried soils. Aust. J. Soil Res. 23:613-621.

Sparling, G. P., C. W. Feltham, J. Reynolds, A. W. West, and P. Singleton. 1990. Estimation of soil microbial C by a fumigation-extraction method: use on soils of high organic matter content and a reassessment of the k_{EC} factor. Soil Bio. Biochem. 22:301-307.

Stanford, F. and S. J Smith. 1972. Nitrogen mineralization potential of soils. Soil Sci. Soc. Am. J. 36:465-472.

Tate, K. R., D. J. Ross, A. J. Ramsay, and K. N. Whale. 1991. Microbial biomass and bacteria in two pasture soils: An assessment of measurement procedures, temporal variations, and the influence of P fertility status. Plant and Soil 132:233-241.

Tiedje, J.M. 1982. Denitrification In Methods of Soil Analysis, Part 2. Chemical and Microbial Properties. Agronomy No. 9 (A.L. Page, R.H. Miller and D.R. Keeney Eds.) 2nd Edn. pp 1011-1026. American Society of Agronomy, Madison.

Trehan, S. P. 1996. Immobilisation of $^{15}NH_4^+$ in three soils by chemical and biological processes. Soil Biol. Biochem. 28:1021-1027.

US Environmental Protection Agency (USEPA) 1983. Methods for chemical analysis of water and wastes. Environ. Monit. Support Lab., Cincinnati, OH.

Vance, E. D., P. C. Brookes, and D. S. Jenkinson, D.S. 1987. An extraction method for measuring soil microbial biomass C. Soil Biol. Biochem. 19:703-707.

Velthof, G. L., S. C. Harvis, A. Skin, A. G. Allen, and O. Oenema. 1996. Spatial variability of nitrous oxide fluxes in mown and grazed grasslands on a poorly drained clay soil. Soil Biol. Biochem. 28:1215-1225.

Vymazal, J., C. B. Craft and C. J. Richardson. 1994. Periphyton response to nitrogen and phosphorus additions in the Florida Everglades. Algological Stud. 73:75-97.

Waksman, S. A. and E. R. Purvis. 1932. The influence of moisture content upon the rapidity of decomposition of lowmoor peat. Soil Sci. 34:323-336.

Wardle, D. A. 1992. A comparative assessment of factors which influence microbial biomass carbon and nitrogen levels in soil. Biol. Rev. 67:321-358.

Waring, S. A. and J. M. Bremner. 1964. Ammonium production in soils under waterlogged conditions as an index of nitrogen availability. Nature 201:951-952.

Wheeler, P. A. and D. L. Kirchman. 1986. Utilization of inorganic and organic nitrogen by bacteria in marine systems. Limnol. Oceanogr. 31:998-1009.

Williams, B. L. 1984. The influence of peatland type and the chemical characteristics of peat on the content of readily mineralizable nitrogen. Proceedings of the 7th International Peat Congress 4:410-418.

Williams, B. L. and G. P. Sparling. 1988. Microbial biomass carbon and readily mineralized nitrogen in peat and forest humus. Soil Biol. Biochem. 20:579-581.

Wu, Y., F.S. Sklar, K.R. Rutchey. 1997. Analysis and simulations of fragmentation patterns in the Everglades. Ecol. Appl. 7:268-276.

Yan, S. 1995. The role of denitrification in nitrate removal from a poorly drained sandy soil irrigated with dairy effluent. Ph.D dissertation, University of Florida, Gainesville, FL. 231 p.

Yoshinari, T. and R. Knowles. 1976. Acetylene inhibition and nitrous oxide reduction by denitrifying bacteria. Biochem. Biophys. Res. Commun. 69:705-710.

BIOGRAPHICAL SKETCH

John White was born in New England where the beans are baked and the lobsters have claws. He lived in Shrewsbury, MA until setting out for college at age 17. John parlayed childhood loves of collecting rocks, combing the ocean shores for treasures, and playing in mud puddles into a B.S. degree in Geology from Washington & Lee University, 2 Master's degrees from the Florida Institute of Technology in the study of Geological Oceanography and Coastal Zone Management, and finally culminating in the receipt of the Ph.D. degree in Soil and Water Science at the University of Florida.

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation is fully adequate, in scope and quality as a dissertation for the degree of Doctor of Philosophy

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